UNIVERSITÉ DE SHERBROOKE
Faculté de génie
Département de génie civil

LES HERBICIDES DANS L'ENVIRONNEMENT AQUATIQUE ET LEURS EFFETS SUR LES COMMUNAUTÉS DE PHYTOPLANCTON LACUSTRES

Marieke BEAULIEU

Sherbrooke (Québec) Canada
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MEMBRES DU JURY

Hubert CABANA
Directeur

Yannick HUOT
co-directeur

Jean-Philippe BELLENGER
Évaluateur

Philippe JUNEAU
Évaluateur

Jay LACEY
Évaluateur

RÉSUMÉ

Globalement, l'agriculture est la plus importante source de pollution des eaux de surface à l'échelle du bassin versant, menaçant la biodiversité aquatique globalement. Les herbicides, la plus grande fraction des pesticides utilisés au Québec (par poids), représentent un risque particulier envers le phytoplancton en raison des similarités de ces derniers avec les plantes terrestres. Les efflorescences de cyanobactéries, et leurs effets néfastes (cyanotoxines, géosmine, épuisement en oxygène lors de leur décomposition), pourraient être favorisés par la contamination par les herbicides.

La majorité des études écotoxicologiques sur les herbicides et le phytoplancton mesurent des effets aigus sur des cultures plutôt que des communautés, à des concentrations rarement retrouvées dans l'environnement. Cette thèse étudie : (1) les effets d'herbicides à des concentrations rencontrés dans l'environnement au Québec, sur des communautés naturelles de phytoplancton lors d'expériences de plus d'une semaine et en (2) modélisant la contamination des lacs par l'atrazine à grande échelle spatiale. L'utilisation de méthodes permettant d'observer la réponse physiologique des organismes améliore notre compréhension des effets subléthaux de ces polluants.

Cette thèse aborde d'abord les effets de l'atrazine et du métolachlor sur des communautés naturelles de phytoplancton lors d'expériences de 3 semaines (1er article). En l'absence d'effets sur la chlorophylle *a* ou la structure de la communauté de phytoplancton, l'exposition au métolachlor, et de moindre façon à l'atrazine, induit des changements dans l'expression des protéines de stress du phytoplancton et, pour le métolachlor, diminue la diversité de communautés bactériennes. Ces résultats suggèrent une acclimatation physiologique du phytoplancton exposé à de faibles concentrations d'herbicides. Le deuxième article étudie les effets d'herbicides sur la photo-physiologie du photosystème II en utilisant le protocole de florescence à taux de répétition rapide lors d'expériences d'une semaine. Il démontre la grande sensibilité de cette méthode, tant pour les cultures d'algues eucaryotes, de cyanobactéries, et des communautés naturelles de phytoplancton. Sans différences notables entre les groupes considérés, la photosynthèse de toutes les cultures et communautés était inhibée à des concentrations inférieures à celles des directives et normes nationales.

Il est difficile d'estimer le risque que représentent les herbicides dans les lacs car ils y sont rarement quantifiés. Dans le chapitre 3, nous abordons l'étendue de la contamination des lacs par les herbicides en développant des modèles prédictifs pour les lacs des États Unis contigus. La contamination par l'atrazine, détectée dans près du tiers des lacs, était avant tout lié à l'utilisation des terres. Selon les résultats des autres chapitres ~10% des lacs américains ont des concentrations d'atrazine susceptibles d'affecter la physiologie du PSII du phytoplancton.

Alors que cette thèse ne suggère pas que les cyanobactéries soient favorisés lors d'exposition à de faibles concentrations d'herbicides, elle démontre que les concentrations présentement retrouvés dans l'environnement entrainent des effets physiologiques des communautés à des concentrations qui n'affectent ni la structure des communautés ou les estimations de biomasse.

Mots clefs : Herbicides, Atrazine, Métolachlor, Phytoplancton, Cyanobactéries, Protéines de stress, Fluorescence à taux de répétition rapide, Modèle spatial

YOU LIVE THE SURPRISE RESULTS OF OLD PLANS

JENNY HOLZER, SURVIVAL (1983-1985)

À mes parents, Louis et Elzeliena, pour votre amour et votre soutien, depuis toujours.

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INTRODUCTION

1.1 Mise en contexte et problématique

Les pesticides sont, par définition, des substances toxiques, utilisées afin d'éliminer des organismes nuisibles à l'activité humaine. L'usage de pesticides à grande échelle dans le milieu agricole, un évènement socioéconomique critique du 20e siècle, constitue une clef de l'agriculture moderne suite de la révolution dite « verte » (Pimentel, 1996). Le secteur de l'agriculture représente la plus grande proportion de ventes de pesticides. Au Québec, en 2016, ce secteur représentait 82 % du volume de ventes (par poids) (MDDELCC 2018).

Les herbicides sont depuis une demi-décennie la méthode de contrôle dominante des mauvaises herbes (Davis et Frisvold, 2017). Dans le secteur agricole québécois, la plupart des pesticides vendus sont des herbicides (68 %), pour une application annuelle estimée de 2,18 kg d'herbicides par hectare de terre cultivée (MDDELCC 2018). Les ventes au Québec ont crû au cours des 20-30 dernières années, phénomène également observé ailleurs en Amérique du Nord (Davis et Frisvold, 2017). Jusqu'à 5 % de la masse appliquée de pesticides peut ressortir des sols agricoles par lessivage, écoulement par drainage et ruissèlement de surface (Carter 2000), avec des pics de concentration dans les ruisseaux environnants à la suite d'évènements de pluie (Bundschuh et al., 2014). Leur usage croissant suggère que la perception des bénéfices économiques liés à l'usage des herbicides continue de supplanter celle du risque environnemental qu'ils peuvent représenter.

1.1.1 Rendements versus environnement

On estime que les substances agrochimiques ont permis de doubler la production agricole au cours du dernier siècle (Carvalho 2017) par l'intensification de l'agriculture, subvenant aux besoins d'une population mondiale croissante. Par exemple, la production céréalière a triplé avec seulement une augmentation de 30 % de la surface de culture (Wik et al., 2008). Leur usage est généralement perçu comme bénéfique par les producteurs. Un investissement de 1 $ en dépenses en pesticides permet d'augmenter la valeur brute de la production de 3 à 6,50 $ (Zilberman et al., 1991). L'envers des bénéfices liés à l'usage de pesticides est le risque qu'ils représentent envers la santé humaine et l'environnement (Carvalho 2017). La publication de *Silent Spring* (Carson, 1962) qui abordait les conséquences environnementales de l'usage du DDT a été un moment séminal pour les mouvements environnementaux, culminant par le ban

de la molécule. Depuis, de nouvelles formulations moins persistantes et estimées sécuritaires ont fait leur entrée sur le marché. Bien que ces composés soient moins persistants et nocifs envers leurs usagers et les milieux environnants que leurs prédécesseurs, les exemples d'effets néfastes cumulent, notamment concernant les effets des insecticides sur les populations d'abeilles (Blacquière et al., 2012, Park et al., 2015) et ceux des herbicides sur les amphibiens (Hayes et al., 2010, Rimayi et al., 2018). Si le patron de l'usage de pesticides se maintient, la production globale de pesticides en 2050 devrait être 2,7 fois les niveaux de 2001 (Tilman et al., 2001). Sous ces conditions, la contamination environnementale par les pesticides continuera de s'amplifier au cours des années à venir.

1.1.2 La vulnérabilité des écosystèmes d'eau douce

Les pesticides d'origine agricole représentent un risque pour les milieux d'eau douce, d'abord par leur proximité. Ces milieux sont également soumis à de nombreuses sources de stress d'origine anthropique (eutrophisation, changements climatiques, espèces envahissantes, pollution). Bien que les milieux d'eau douce aient un grand potentiel d'adaptation à des facteurs de stress multiples (Jackson et al., 2015) et que certains organismes y habitant aient la capacité de s'adapter à la présence de certains pesticides (Cothran et al., 2013), les espèces d'eau douce subissent des déclins de populations de 83 % depuis 1970 (WWF 2018). Le risque d'extinction de ces espèces est supérieur à celui d'espèces terrestres (Collen et al., 2013). Les milieux d'eau douce occupent moins de 1 % de la surface terrestre, mais accueillent environ 10 % des espèces connues (Strayer et Dudgeon, 2010). En plus de la valeur évidente de l'eau douce pour la multitude de services rendus (Green et al., 2015), il est pertinent de s'intéresser à la santé de ces écosystèmes en raison de leur grande vulnérabilité (Vörösmarty et al., 2010, Rodell et al., 2018).

Plusieurs études s'intéressent aux effets des pesticides dans l'environnement aquatique. En contrepartie, on connaît peu le sort des pesticides et autres molécules d'intérêt émergent dans l'environnement aquatique (Gavrilescu et al., 2015, Brack et al., 2017). On tente actuellement de cartographier ces molécules dans l'eau souterraine et l'eau potable (Lopez et al., 2015 ; Machado et al., 2016 ; Torres et al., 2017, Glassmeyer et al., 2017) et dans les ruisseaux et rivières (Kolpen et al., 2002; Loos et al., 2009 ; Munoz et al., 2015 ; Bradley et al., 2017 ; Elliott et al., 2017). Comparativement, il existe peu de publications sur la contamination des

lacs par ces molécules, bien que contamination temporelle des lacs de grande surface est relativement bien représentée (Larras et al., 2016, Xie et al., 2015). En contrepartie, l'étude de la contamination des lacs à l'échelle du paysage est beaucoup plus récente (EPA-NLA 2016 ; Berman et al., 2018).

1.1.3 Cyanobactéries, agriculture et herbicides

Un phénomène exacerbé par l'usage d'engrais et la perte d'intégrité des sols à grande échelle par l'agriculture est l'eutrophisation anthropique. Il est généralement établi que les conditions de nutriments élevés sont favorables à la prolifération des cyanobactéries, formant des efflorescences parfois toxiques (Downing et al., 2001). L'évidence cumule sur l'augmentation de la fréquence, magnitude et durée des efflorescences de cyanobactéries (Huisman et al., 2018). Bien que la pollution diffuse soit plus difficile à contrôler que celle provenant de sources ponctuelles (p. ex. stations de traitement des eaux usées), le contrôle de la pollution par le phosphore a permis de ralentir le rythme d'eutrophisation de bon nombre d'écosystèmes aquatiques (Schindler 2012). Parmi les multiples facteurs additionnels pouvant favoriser les espèces cyanobactériennes, les effets des pesticides sont généralement peu discutés (Dokulil et Teubner, 2000 ; Harris et Smith 2016). Les pesticides, plus particulièrement les herbicides qui, par leur phytotoxicité, pourraient affecter de façon différentielle les groupes du phytoplancton. Leurs effets dans l'environnement sont difficiles à distinguer de ceux des nutriments d'origine agricoles. Bien qu'il y ait une documentation abondante des effets des herbicides sur les communautés de microalgues lors d'expériences (voir État de l'Art), ces études n'offrent pas de réponse claire sur les effets probables des herbicides sur la dominance cyanobactérienne. On y retrouve des effets variables et parfois contradictoires. De plus, les concentrations testées sont souvent supérieures à celles observées dans l'environnement (Relyea et Hovermann 2006).

1.2 Question de recherche

Cette thèse cherche à déterminer si les concentrations d'herbicides dans l'environnement peuvent favoriser les efflorescences de cyanobactéries. Pour ce faire, il est d'abord nécessaire de quantifier la présence de pesticides dans l'environnement. Puis il est nécessaire de considérer les effets de ces concentrations d'herbicides sur des communautés naturelles de

phytoplancton en tentant de simuler des conditions représentatives de l'environnement et en considérant des mesures structurelles et fonctionnelles variées et susceptibles d'être affectées par les herbicides à ces concentrations. Finalement, le développement de modèles empiriques permet d'étendre les connaissances acquises à une plus grande échelle spatiale.

1.3 Contributions originales

Le volet expérimental de cette thèse s'insère dans le courant de la toxicologie des systèmes (Waters & Fostel, 2004). Les avancées technologiques changent la manière dont l'écotoxicologie est pratiquée ; certains y voient même une « révolution de l'écotoxicologie » (Garcia-Reyero & Perkins 2011). Ce domaine est en plein essor, démontrant actuellement les effets directs (et transgénérationnels [Kubsad et al., 2019]) des contaminants sur la physiologie des vertébrés (Ali et al., 2018 ; Bautista et al., 2018 ; Pereira et al., 2018 ; Orton et al., 2018) et invertébrés (Awali et al., 2019), mais également chez le phytoplancton (Filimonova et al., 2016, Esperanza et al., 2017 ; Demailly et al., 2019).

Dans le Chapitre 3, nous investiguons les effets de l'atrazine et du métolachlor à des concentrations retrouvées dans l'environnement sur une variété de paramètres fonctionnels et structurels et utilisons la protéomique pour déterminer si cette exposition a des effets sur l'expression des isoformes de protéines de stress. Bien que reconnus comme biomarqueurs de pollution, les protéines de stress n'ont jamais été étudiées chez les communautés de phytoplancton exposées à des herbicides.

La fluorescence du photosystème II est reconnue depuis quelque temps déjà comme bio-indicateur des effets de contaminants sur la physiologie de producteurs primaires (El Jay 1996, Juneau et Popovic 1999 ; Dorigo et al., 2001). En contrepartie, l'utilité des paramètres spécifiques au paramètre du protocole de fluorescence à taux de répétition rapide (FRRF) est investiguée pour la première fois ici dans les Chapitres 3 et 4. En plus d'étudier ces paramètres physiologiques pour une variété de classes d'algues eucaryotes et de cyanobactéries, nous testons son applicabilité aux communautés naturelles de phytoplancton. Étant donné que la méthode est simple, rapide et non invasive, elle pourrait avoir une utilité considérable comme bio-indicateur et outil de terrain.

Finalement, nous analysons les données d'atrazine du EPA National Lake Assessment (U.S. EPA. 2016) qui a quantifié l'atrazine dans plus de 1000 lacs aux États-Unis. À notre connaissance, il n'existe pas de modèles prédictifs d'atrazine pour les lacs. Le développement de ces modèles permet d'évaluer le risque associé à la pollution par les herbicides à grande échelle spatiale. En plus d'identifier les facteurs affectant la contamination des lacs par l'atrazine, de tels modèles permettront d'étaler nos connaissances de l'état de contamination vers des systèmes qui ne sont pas échantillonnés.

1.4 Plan du document

Dans une première partie (Chapitre 2), une brève revue de la littérature traitant des effets des herbicides sur les communautés de phytoplancton est présentée.

Dans le Chapitre 3, nous présentons les données recueillies au cours des campagnes de terrain, présentant les concentrations d'herbicides et d'autres molécules d'intérêt émergent ont été quantifiées dans le lac Massawippi, Cantons-de-l'Est (Québec) et son bassin versant. Nous présentons les résultats d'expériences de trois semaines sur des communautés de phytoplancton du lac Massawippi. Testant des concentrations d'atrazine et de métolachlor qui sont vraisemblablement retrouvés dans l'environnement aquatique, nous étudions leurs effets sur la structure des communautés de phytoplancton, la biomasse (estimée par la fluorescence de la chlorophylle *a* extraite), les paramètres FRRF, la diversité des communautés bactériennes associées aux différentes fractions de taille de la communauté (par analyse16S rARN) et la diversité des isoformes de protéines de stress.

Dans le Chapitre 4, nous testons les effets de l'atrazine, du métolachlor et du DCMU (Diuron) à diverses concentrations lors d'expériences d'une semaine sur des cultures de cyanophycées, bacillariophycées, chlorophycées et cryptophycées ainsi que sur des communautés naturelles prélevées dans deux lacs. Nous déterminons les concentrations auxquels des effets sur les paramètres FRRF sont observés et comparons ces valeurs entre les différentes cultures et les communautés naturelles. Ces concentrations sont également comparées à celles auxquelles nous observons des effets sur la fluorescence de la chlorophylle *a* extraite ainsi que les dénombrements cellulaires.

Dans le Chapitre 5, nous déterminons quel risque représentent les concentrations d'atrazine retrouvées dans les lacs des États-Unis en développant des modèles empiriques prédisant la concentration à grande échelle spatiale dans plus de 1000 lacs choisis pour être représentatifs de tous les lacs du pays. Ces modèles font le lien entre les résultats observés lors de nos expériences et la contamination réelle des eaux de surface.

CHAPITRE 2

L'ÉTAT DE L'ART

Les effets des herbicides sur les communautés de phytoplancton sont bien étudiés et une revue exhaustive n'est pas présentée ici. Ce chapitre s'intéresse seulement aux effets de trois herbicides spécifiques : l'atrazine, le glyphosate et le métolachlor. Ces molécules représentent la plus grand proportion de ventes d'herbicides au Québec et ils sont les plus fréquemment détectés dans les cours d'eau dans la province (Giroux et al. 2015) Il porte aussi sur les autres facteurs pouvant moduler les effets des herbicides, notamment les mélanges, l'environnement physico-chimique, et la considération d'autres niveaux trophiques.

2.1 Herbicides et communautés phytoplanctoniques

2.1.1 Atrazine

a) Effets sur la biomasse des communautés

L'herbicide le plus étudié en termes de ses effets sur le phytoplancton est sans doute l'atrazine (Solomon et al., 1996 ; DeLorenzo et al., 2001). Tel que synthétisé par Pesce (2011) les concentrations en atrazine de 20 à 1000 $\mu g\ L^{-1}$ causent des diminutions dans les densités de phytoplancton estimées par la chlorophylle *a*. Ces concentrations sont inférieures aux concentrations maximales observées dans l'environnement québécois (11 $\mu g\ L^{-1}$; Giroux 2015). À des concentrations moindres, les effets observés sont plus variables. Certaines études n'observent aucun effet à des concentrations de 5 à 14 $\mu g\ L^{-1}$ (van den Brink et al., 1995 ; Leboulanger et al., 2001 ; Pinckney et al., 2002 ; Relyea, 2009) alors que d'autres observant la stimulation de la chlorophylle *a* à des concentrations de 1 à 20 $\mu g\ L^{-1}$ (Gustavson et Wängberg, 1995 ; Seguin et al., 2002). Ces dernières observations sont possiblement dues à l'augmentation de la concentration de chlorophylle *a* intracellulaire lorsque le phytoplancton est exposé à de faibles concentrations d'atrazine par une acclimatation similaire à celle en présence de faible luminosité (p. ex. Lürling et Roessink, 2006 ; Feckler et al., 2018). Dans ce cas, l'absence d'effets observés dans les premières études pourrait être accompagnés par une diminution du nombre de cellules, ou d'une diminution de la taille cellulaire.

b) Effects sur les communautés sans cyanobactéries

Weiner et al. (2004) ont trouvé qu'il y avait une corrélation significative entre l'assimilation d'atrazine par les microalgues et leur sensibilité, les plus petits organismes avec une plus grande surface volumique étaient plus sensibles. Certaines études démontrent que l'atrazine module les structures des communautés de phytoplancton. Schmitt-Jansen et Altenburger (2005) ont étudié les effets de l'atrazine, de l'isoproturon et de la prométryne sur une communauté de périphyton. Ils ont observé que l'exposition à ces pesticides mène à une dominance des bacillariophycées ainsi qu'une perte de biomasse et de biodiversité.

DeNoyelles et al. (1982) trouvèrent que 20 $\mu g\ L^{-1}$ d'atrazine réduisait la croissance du phytoplancton après quelques jours suivi d'un changement dans la succession du phytoplancton avec l'établissement de chrysophycées (*Mallomonas*) et des cryptophycées (*Cryptomonas*). Les espèces subissant des déclins à haute concentration étaient des pyrrhophytes et des chlorophycées. Hamilton et al. (1988) ont observé que 100 $\mu g\ L^{-1}$ d'atrazine diminuaient la diversité des communautés de phytoplancton, par la perte des chlorophycées, qui étaient presque exclusivement les organismes affectés. Jüttner et al. (1995) trouvèrent que l'atrazine affecta les communautés à des concentrations de 182 $\mu g\ L^{-1}$ dans des étangs expérimentaux. Les bacillariophycées étaient les moins sensibles, mais les cryptophycées dominèrent à l'automne aux concentrations intermédiaires testées (68 $\mu g\ L^{-1}$ et moins). Hoagland et al. (1993) trouvèrent que l'atrazine réduisait significativement la concentration en chlorophylle *a* ainsi que la turbidité, mais n'eut pas d'effets significatifs sur la biomasse ou les dénombrements cellulaires. À plus long terme, ils observèrent cependant une diminution des colonies d'algues vertes et de certains microinvertébrés. Kasai et Hanazato (1995) ont étudié les effets de la simétryne, un autre herbicide triazine à 100 $\mu g\ L^{-1}$. L'herbicide impacta négativement la biomasse des cryptophycées et chlorophycées.

Bien que ces communautés ne comptaient pas de représentants des cyanobactéries, il semblerait que les chlorophycées soient généralement plus sensibles aux effets de l'atrazine alors les cryptophycées et les bacillariophycées pourraient bénéficier des conditions liées à la présence d'atrazine comparativement aux autres groupes jusqu'à une certaine concentration environnementale de l'herbicide. Les effets de l'atrazine pourraient cependant être négligeables dans l'environnement. Andrus et al., 2015 ont démontré qu'en tenant compte

d'autres facteurs (p.ex., chimie, géographie, hydroclimatologie), la contamination en atrazine n'explique pas plus que 2,2 % de la variance en diversité et abondance des microalgues dans les ruisseaux du Midwest des États-Unis.

c) Effets sur les communautés avec cyanobactéries

Parmi les études sur les communautés incluant des cyanobactéries, Yates et Rogers (2011) ont trouvé que les cyanobactéries *Gleocapsa sp., Oscillatoria sp.* et *Nostoc sp.* étaient, avec *P. parvum* et *Cryptomonas sp*., les organismes les plus tolérants au traitement de 10 $\mu g\ L^{-1}$. Ils étaient plus tolérants que les autres phototrophes obligatoires et certains mixotrophes (*Mallomonas sp*., *Ochromonas sp. Euglena sp.)*. Zananski et al. (2010) ont étudié les effets de l'atrazine (0, 5, 20 et 50 $\mu g\ L^{-1}$) et trouvèrent que les cyanobactéries diminuèrent avec l'augmentation de la concentration en atrazine en début d'été (juin, juillet), mais augmentèrent de façon concomitante avec la concentration en atrazine en août, suggérant une augmentation de la tolérance de la communauté ou des différences de tolérance des espèces retrouvées lors de la succession saisonnière. Séguin et al. (2002) ont, pour leur part trouvé que 30 $\mu g\ L^{-1}$ d'atrazine avaient un impact négatif significatif sur la chlorophylle *a* et le poids sec du phytoplancton, avec une diminution des cyanobactéries dans les communautés.

2.1.2 Glyphosate

Le glyphosate a également été le sujet d'un nombre considérable d'études portant sur les effets sur les communautés. Smedbol et al. (2017) ont trouvé que le glyphosate avait des effets sur le photosystème II des cyanobactéries et algues eucaryotes, mais que cette molécule affectait d'avantages les taux de croissance que la photosynthèse. La molécule peut altérer la structure et la fonction des communautés de phytoplancton (Smedbol et al. 2018). Certaines études trouvèrent que le glyphosate stimula la biomasse et la production primaire (Schaffer et Sebetich 2004, Relyea 2005). Pesce et al. (2009) ont trouvé que les bacillariophycées étaient favorisées par la molécule. Les cyanobactéries étaient absentes de cette étude. Abdel-Hamid et al. (1996) rapportent également que le glyphosate diminue la diversité d'organismes. Les chlorophycées étant les plus affectées. D'autres études rapportent des diminutions de la biomasse totale à de faibles concentrations de glyphosate. Les concentrations de glyphosate requises pour induire des diminutions de biomasse sont considérables, mais pourraient favoriser les cyanobactéries et les chlorophycées (8 $mg\ L^{-1}$, Vera et al., 2009). Le glyphosate,

dont 14 % du poids moléculaire est constitué de phosphate, était une source d'eutrophisation à la fin de l'expérience. Cette capacité d'utiliser le glyphosate comme source de nutriments n'était pas congruente avec la phylogénie des espèces (Wang et al., 2016). Certaines études observent que le glyphosate stimule les cyanobactéries (Saxton et al., 2011, Vera et al., 2012) et les picocyanobactéries (Pérez et al., 2007). Selon Yates et Rogers (2011), des concentrations de 100 $\mu g\ L^{-1}$ de glyphosate avaient des impacts négatifs sur les cyanobactéries, mis à part *Oscillatoria sp.*, qui comptait parmi les organismes plus tolérants. Dans les formulations commerciales de glyphosate (Roundup®), l'isopropylamine, une des trois composantes principales, était plus toxique que le glyphosate envers les espèces phytoplanctoniques (Lipok et al., 2010).

Bien que les résultats de études rapportés ne soient pas unanimes, il semblerait que le glyphosate serait une molécule pouvant favoriser les efflorescences cyanobactériennes, du moins pour certains taxons. Cette réponse pourrait être due en partie à l'enrichissement en phosphore lié à la dégradation de la molécule par les bactéries hétérotrophes (Saxton et al. 2011). Les concentrations où des effets sont observés sont cependant relativement élevées, dépassant largement les concentrations maximales observées dans l'environnement québécois (18 $\mu g\ L^{-1}$; Giroux 2015).

2.1.3 Métolachlore

Relyea (2009) n'a observé aucun effet du métolachlore (7 $\mu g\ L^{-1}$) sur la biomasse ni sur la chlorophylle *a* d'une population de périphyton suite à 16 ou 35 jours d'exposition. Debenest et al. (2009) ont étudié les effets du métolachlore sur des communautés de bacillariophycées et ont démontré qu'une concentration de 30 $\mu g\ L^{-1}$ de métolachlore n'avait aucun effet sur l'abondance de la bacillariophycée *Melosira varians* après 3 jours d'exposition alors que des effets étaient ressentis sur d'autres espèces de ce groupe à des concentrations de 5 à 30 $\mu g\ L^{-1}$. Spawn et al. (1997) ont étudié les effets de l'alachlore sur les communautés de périphyton en microcosmes. À des concentrations de plus de 1 $\mu g\ L^{-1,}$ il y eut un effet négatif sur la biomasse totale. À des concentrations de plus de 30 $\mu g\ L^{-1,}$ il y eut un changement dans les espèces dominantes, avec une augmentation des chlorophycées filamenteuses, *Fragilaria crotensis* (Bacillariophycée) et les cyanobactéries unicellulaires, mais les différences n'étaient pas statistiquement significatifs pour ces derniers. Les effets de cette famille de pesticides

devraient être étudiés davantage afin de cerner des effets sur les cyanobactéries. Alors qu'une concentration de 41 $\mu g\ L^{-1}$ à déjà été recensée dans l'environnement aquatique québécois (Giroux 2002), la concentration maximale observée entre 2012 et 2015 était de 9.9 $\mu g\ L^{-1}$ (Giroux 2015).

2.2 Effets des mélanges de pesticides sur le phytoplancton

Dans l'environnement, les organismes sont exposés à des mélanges de pesticides plutôt qu'à des composés uniques (Müller et al., 2002 ; Irace-Guigand et al., 2004 ; Chèvre et al., 2006). Sous l'influence de ces mélanges, les effets sur les communautés biotiques peuvent être additifs (ne dépendent pas des autres), antagonistes (agissent en directions opposées) ou synergiques (créent un effet plus grand que la somme des effets de chacun). Alors que très peu d'études ont, à ce jour, évalué la toxicité combinée des pesticides (Backhaus et al., 2004), les études existantes suggèrent que ceux ayant des modes d'action similaires auraient des effets additifs (Faust et al., 2001, Junghans et al., 2003, Backhaus et al., 2004, Chèvre et al., 2006, Cedergreen et al., 2007, Pesce et al., 2010). Pour les mélanges à mode d'action différents, le modèle additif demeure le plus probable (77 études sur 85, Deneer et al., 2000 ; Knauert et al., 2008, 2009, Lozano et al., 2018), mais certains trouvent des effets antagonistes (Cedergreen et al., 2007). Relyea (2009) a trouvé que pour le phytoplancton, les impacts des mélanges pouvaient être largement prédits par les impacts des pesticides individuels, mais que ce n'est pas nécessairement le cas pour d'autres types d'organismes (dans leur cas les amphibiens). Gregorio et al. (2012) ont, par contre, observé qu'un mélange de pesticides avait plus d'effets sur l'abondance des espèces phytoplanctoniques que ce qui serait prédit par l'addition de leurs concentrations respectives. Peu d'études se sont intéressées aux effets des mélanges d'herbicides sur les cyanobactéries (Ye et al., 2013).

Dorigo et al. (2007) ont étudié les effets d'un mélange de pesticides sur des communautés benthiques de microalgues et de cyanobactéries. Ils observèrent une diminution de la biomasse, mais un maintien de la richesse d'espèces, caractérisé par un remplacement des espèces chlorophycées par des bacillariophycées. Gregorio et al. (2012) ont analysé des données environnementales afin de déterminer les impacts de 14 pesticides sur le phytoplancton du lac de Genève (Suisse) et ont déterminé que la toxicité des mélanges d'herbicides était un paramètre clé pour expliquer les changements dans l'abondance

d'espèces de phytoplancton dans ce lac. Les contributeurs majeurs à la toxicité étaient le phénylure, le monolinuron et le diuron. Knauer et al. (2010) ont étudié l'effet d'un mélange d'atrazine, d'isoproturon et de diuron sur une communauté pélagique. Le mélange causa une diminution de la chlorophylle *a* et de la biomasse totale. Les groupes les plus sensibles au mélange étaient les cyanobactéries, les cryptophycées et les bacillariophycées, sensibilité qui semble favoriser la dominance des chlorophycées. Les cyanobactéries étaient particulièrement sensibles à l'atrazine. Lizotte et al. (2012) ont développé une expérience *in situ* où un mélange de nutriments, d'atrazine, de métolachlore et de perméthrine était déversé dans un marais simulant l'effet d'un effluent agricole suite à de fortes pluies. La diminution de la productivité totale était plus forte près du site de déversement, mais était détectée jusqu'à 40 m en aval, et ce, jusqu'à 14 jours après l'événement. Les effets de l'atrazine (12 et 150 μg L^{-1}) et l'alachlore (5 et 90 μg L^{-1}) seraient additifs plutôt que synergiques et causèrent une diminution des biovolumes cellulaires d'algues benthiques pendant quatre semaines (Carder et Hoagland 1998). *Cylindrospermum majus*, une cyanophycée, faisait partie des six espèces dominantes sur lesquelles ils ont étudié les effets du pesticide, mais son abondance relative au sein de la communauté n'a pas changé au cours de l'expérience.

Selon ces études, les cyanobactéries ne semblent pas être favorisées lorsqu'exposées à des mélanges d'herbicides. Cela pourrait être dû au petit nombre d'études recensées, mais pourrait également être une sensibilité accrue lorsqu'exposés à des stress multiples. Des mélanges de molécules ayant différents modes d'action mériteraient d'être davantage étudiés à l'avenir.

2.3 Effets des stress multiples

Alors que les mélanges de pesticides peuvent être considérés comme des stress multiples, les milieux d'eau douce sont sujets à d'additionnels agents de stress tels que l'eutrophisation, la présence d'espèces envahissantes et les changements climatiques. Il est difficile d'isoler les effets propres aux pesticides dans des systèmes naturels sujets à des sources de variation multiples. Dès lors, il est impératif d'utiliser des expériences en laboratoire (microcosme) et sur le terrain (mésocosme) afin d'isoler les effets spécifiques de chaque agent et de déterminer l'effet combiné de stress.

Parmi les scénarios de stress multiples à considérer les nutriments, la température et la luminosité pourraient moduler l'effet des pesticides sur les populations phytoplanctoniques (Bérard et al., 1999 ; Chalifour et Juneau, 2011 ; Lizotte et al., 2012 ; Deblois et al., 2013). Guasch et al. (1998 b) ont étudié la relation entre les variables environnementales sur la composition de la communauté et la sensibilité du périphyton à l'herbicide atrazine dans 20 ruisseaux et rivières sur un gradient latitudinal (Suède, Pays-Bas, Espagne) utilisant une analyse d'ordination et ont démontré que la sensibilité à l'atrazine, déterminée par les CE_{50} des communautés, était corrélée de façon positive avec la lumière et le pourcentage des différents groupes d'organismes présente. Cette étude suggère que les bacillariophycées seraient avantagées par la présence d'atrazine. Gomes et Juneau (2017), dans une revue des interactions entre la lumière, température et les herbicides, ont trouvé que la température était le facteur le plus important dans la modulation de la toxicité des herbicides.

2.3.1 Pesticides et saisonnalité

La résistance aux pesticides du phytoplancton au cours de la saison libre de glaces semble variable. Bérard et al. (1999) ont trouvé que la sensibilité à l'atrazine diminuait avec une augmentation de la température de l'eau. Ces effets sont cependant difficiles à isoler des changements saisonniers dans la communauté phytoplanctonique. Ces résultats sont appuyés par Chalifour et Juneau (2011) qui ont observé qu'une diminution de la température peut réduire la tolérance aux pesticides. La capacité d'augmenter la concentration en pigments caroténoïdes protecteurs et de dissiper l'énergie non photochimique à ces températures confère une protection aux effets de l'atrazine aux bacillariophytes (Chalifour et Juneau, 2011).

Bérard et Benninghoff (2001) ont trouvé que l'atrazine affectait *Oscillatoria limnetica* en communauté de façon différente au cours de la saison libre de glaces. La croissance d'*Oscillatoria limnetica*, était inhibée (-95 %) en mars et en mai, inchangée en juillet et stimulée en septembre (+98 %). Leurs résultats suggèrent que les effets de l'atrazine peuvent varier de façon saisonnière de façon concomitante avec la qualité de l'eau et les conditions physiques et climatiques. Les effets sélectifs de l'atrazine seraient également affectés par la biomasse et la composition d'espèces selon les auteurs. Selon ces études, la présence d'herbicides en fin d'été pourrait créer des conditions avantageant les cyanobactéries. Cela

suggère qu'il serait important d'avoir un meilleur suivi de ces molécules et de leurs produits de dégradations au cours de la saison.

2.3.2 Pesticides et luminosité

Alors que la lumière est une ressource essentielle pour le phytoplancton, les optimums de luminosité diffèrent entre les organismes et différents climats de lumière peuvent entraîner des effets tels le stress oxydatif ou l'inhibition de la photosynthèse. Certaines études ont trouvé qu'une forte luminosité augmente l'inhibition par les herbicides (Millie et al., 1992 ; Chen et al., 2012). L'exposition à des inhibiteurs du photosystème II simule des conditions de faible luminosité auxquels les organismes s'acclimatent en générant un plus grand nombre de centres de réaction (Escoubas et al. 1995). Conséquemment, lorsqu'exposés à une forte luminosité, l'excedent de centres de réactions pourrait mener à la création d'un plus grand nombre espèces d'oxygène réactifs. Deblois et al. (2013) ont eux aussi relevé des effets de la luminosité suggérant que lorsque les concentrations en atrazine augmentent avec la turbidité, suivant de fortes pluies par exemple, le phytoplancton en général pourrait être partiellement protégé par les conditions lumineuses faibles. Leurs résultats démontrent également que les espèces cyanobactériennes adaptées à des conditions de lumière élevée sont moins sensibles aux effets des pesticides comparativement aux espèces adaptées à des conditions de faible luminosité. Guasch et al. (1998a, 2003) ont cependant trouvé que l'acclimatation à faible luminosité protégeait de l'exposition à l'atrazine. Ces effets varient potentiellement selon les critères de luminosité considérés

2.3.3 Pesticides et nutriments

Les nutriments sont une ressource essentielle au phytoplancton, reconnue comme étant capable de restructurer les communautés. La co-sélection des espèces phytoplanctoniques par les pesticides et des nutriments a été abordée dans la littérature. Pratt et Barreiro (1998) ont trouvé que la récupération de l'activité du système de transport d'électrons après exposition au diquat était favorisée par des concentrations élevées en nutriments. Les cyanobactéries étaient particulièrement affectées. Ces effets antagonistes entre les nutriments et les herbicides sont également observés pour des communautés estuariennes (Starr et al., 2016).

Wendt-Rasch et al. (2004) ont étudié les effets de l'asulame et le métamitron (herbicides) ainsi que ceux du fluazinam (fongicide) et la cyhalothrine (insecticide) sur des communautés en mésocosmes extérieurs. Leurs résultats suggèrent que les effets des pesticides varient selon le régime trophique du système et que les milieux eutrophes pourraient devenir davantage turbides par la perte de macrophytes sensibles aux herbicides,, engendrant des conditions de faible luminosité favorables à certaines cyanobactéries.

Murdock et Wetzel (2011) ont étudié les effets des mélanges de nutriments et d'atrazine sur des communautés en biofilm en utilisant la microspectroscopie infrarouge et observèrent une augmentation des bandes de phosphodiester et de carbohydrates attribués à l'assimilation non photosynthétique de carbone suite à l'inhibition du photosystème II. La plus grande disponibilité d'azote par l'enrichissement en nutriments serait bénéfique à cette assimilation. Le contenu en protéines était stimulé dans le mélange par rapport aux nutriments seuls, ce qui peut être le résultat d'une stimulation du métabolisme ou le résultat d'une augmentation des pigments phycocyanines en présence de l'inhibiteur de la photosynthèse. Ces résultats suggèrent que les organismes mixotrophes seraient favorisés par les ajouts concomitants de nutriments et d'inhibiteurs de la photosynthèse.

2.3.4 Substances allélopathiques cyanobactériennes

Un autre effet allélopathique potentiellement important, mais qui n'est pas abordé dans la littérature traitant des effets des pesticides est l'effet des cyanotoxines sur les autres espèces d'algues. Les cyanobactéries peuvent induire un stress oxydatif par la production de microcystines et autres composés toxiques. Par exemple, Bártová et al. (2011) ont étudié les effets des microcystines pures et les mélanges complexes de métabolites cyanobactériens sur le stress oxydatif et la détoxification de chlorophycées. Les effets biochimiques des mélanges sur les cultures de chlorophytes étaient hautement variables et différents de ceux des microcystines pures. Les effets combinés de cyanotoxines et de pesticides sur les autres algues dans les communautés de phytoplancton sont peu connus, mais pourraient agir de façon synergique. Étant donné que la présence de pesticides peut moduler le ratio des toxines et la perméabilisation des membranes (ce qui favoriserait le relargage des cyanotoxines) (Polyak et al., 2013), les pesticides pourraient affecter l'allélopathie exercée par les cyanobactéries.

2.4 Considération d'autres niveaux trophiques

Les effets des pesticides sur le phytoplancton peuvent varier selon les interactions avec les niveaux trophiques supérieurs. Les herbicides pourraient rendre certains organismes plus vulnérables au broutage. (Muñoz et al., 2001, Lürling 2011, Zhu et al., 2016). En contrepartie, la réduction de broutage par l'exposition simultanée aux herbicides et aux insecticides ou des effets négatifs d'herbicides sur les niveaux trophiques supérieurs pourraient diminuer l'effet des herbicides sur le phytoplancton (van den Brink et al., 2009, Relyea 2005). Les effets des niveaux trophiques supérieurs sont rarement pris en considération dans les expériences traitant des effets des pesticides. Alors que ces études sont plus difficiles à effectuer, ils sont plus comparables aux interactions existantes dans l'environnement. Étant donné que les cyanobactéries sont généralement considérées peu comestibles étant donné leur présence sous forme de colonies et de filaments, il est pertinent d'aborder la question du broutage dans des communautés. En contrepartie, les herbicides peuvent avoir des effets délétères sur le zooplancton (Hasenbein et al., 2017b). Les communautés bactériennes, elles, pourraient réduire la toxicité des pesticides envers le phytoplancton (Fouilland et al., 2018).

2.5 La fluorescence de la chlorophylle en écotoxicologie

L'utilisation de la fluorescence de la chlorophylle in vivo dans l'étude des effets des herbicides sur les producteurs primaires est bien documentée. Miles et Daniels (1973), utilisant un système de filtres de couleur ont détecté par photographie une augmentation de la fluorescence des plantes, lorsqu'exposées à certains inhibiteurs, notamment la simazine et le diuron. Ahrens et al. (1981) ont démontré qu'une augmentation de la fluorescence n'était pas observée lorsque les mauvaises herbes étudiées étaient résistantes aux herbicides triazines. Richard et al. (1983) ont déterminé que l'utilisation de la méthode flourométrique permettait l'observation d'effets bien avant des symptômes visibles de dommage aux plantes traités aux herbicides inhibiteurs de transport d'électrons.

Ducruet et al. (1984) utilisèrent les cinétiques d'induction de la fluorescence pour démontrer que l'inhibition partielle du photosystème II par le diuron affectait de manière prédictible les paramètres décrivant l'induction de la fluorescence de la chlorophylle. Ces effets furent par la

suite utilisés pour décrire les mécanismes d'action des herbicides (Lazar et al. 1997; Christensen et al. 2003). La sensibilité de la réponse de fluorescence des microalgues en a fait un attrayant biosenseur potentiel de pollution aux herbicides inhibiteurs du PSII (Weston et Robinson 1991; Merz et al. 1996; Védrine et al. 2003 ; Muller et al. 2008).

Bien que l'étude des cinétiques d'induction demeure utilisée pour mesurer les effets des herbicides (Søbye et al. 2010 ; Chalifour et al. 2010), le protocole de fluorescence le plus utilisé en écotoxicologie devint rapidement la fluorométrie par modulation d'impulsion en amplitude (PAM) (Arsalane et al. 1993; Conrad 1993). Cette méthode servit extensivement pour quantifier les effets d'herbicides sur les algues et microalgues (p. ex. Ralph 2000 ; Juneau et al. 2001 ; Frankart et al. 2003 ; Fai et al. 2007 ; Küster & Altenburger 2007; Dewez et al. 2008, Chalifour et al. 2010) et les communautés de microalgues (p. ex. Dorigo et Leboulanger 2001; Séguin et al. 2001 ; Séguin et al. 2002; Schmitt-Jansen & Altenburger 2007, Schmitt-Jansen & Altenburger 2008). Ralph et al. (2007) ont déterminé que parmi les essais biologiques écotoxicologiques basés sur la fluorescence, le rendement quantique maximal (Fv/Fm) et le rendement quantique effectif (ΦPSII) étaient les paramètres le plus sensibles aux effets de contaminants. Bien que le protocole de fluorométrie à taux de répétition rapide fut développé durant ce temps (Kolber et al. 1998), en contraste avec le protocole de fluorescence PAM, il n'est pas rapporté comme protocole de fluorométrie lors d'expériences écotoxicologiques sur les herbicides.

2.6 Conclusions

Alors que la majorité des études démontrent que les herbicides ont divers effets sur les différentes espèces testées et peuvent restructurer les communautés à des concentrations qui sont rencontrées dans l'environnement, la littérature actuelle est insuffisante pour déterminer si les herbicides impactent l'abondance relative des cyanobactéries et crée des conditions propices aux efflorescences. Cet état des faits découle d'une part du nombre relativement restreint d'études et d'autre part de la variabilité des réponses observées dans ces études. D'une revue plus exhaustive de la littérature traitant des effets des herbicides sur le phytoplancton (Beaulieu et al., 2014), 38 % n'étudiaient pas des communautés d'algues où les cyanobactéries représentaient une fraction suffisante de la biomasse totale ou bien ne traitaient

pas d'effets sur la structure des communautés. Du 62 % des études qui pouvaient relever des effets sur les cyanobactéries, 34 % observèrent des effets inhibitoires, 25 % des effets positifs, 25 % des effets variables, c'est-à-dire que la réponse variait selon les conditions biotiques et abiotiques du milieu et 16 % présentaient des résultats neutres.

La littérature scientifique démontre que les communautés phytoplanctoniques sont altérées par la présence de pesticides. Par contre, il n'est pas possible de faire ressortir, à la lumière de ces études, une tendance (positive ou négative) entre la présence de pesticides dans l'eau et celle de cyanobactéries, car plusieurs facteurs expérimentaux ou naturels peuvent influencer les résultats.

CHAPITRE 3

ACCLIMATATION AU STRESS INDUIT PAR LES HERBICIDES PAR LES COMMUNAUTÉS DE PHYTOPLANCTON

Avant-propos

Auteurs et affiliation :

- M. Beaulieu : étudiante au doctorat, Université de Sherbrooke, Faculté de génie, Département de génie civil.
- Y. Huot : professeur, Université de Sherbrooke, Faculté des lettres et sciences humaines, Département de géomatique appliquée
- D. Colatriano : étudiant au doctorat, Université Concordia, Faculté d'arts et sciences, Département de biologie
- S. Kraemer : chercheuse postdoctorale, Université Concordia, Faculté d'arts et sciences, Département de biologie
- H. Cabana : professeur, Université de Sherbrooke, Faculté de génie, Département de génie civil

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Titre anglais : Stress acclimation in phytoplankton communities exposed to metolachlor and atrazine at environmentally relevant concentrations

Contribution au document : Cet article contribue à la thèse en démontrant que des concentrations faibles de métolachlor induisent des changements dans l'expression des protéines de stress dans des communautés naturelles de phytoplancton. Les effets physiologiques subléthaux des herbicides sur le phytoplancton sont peu étudiés en écotoxicologie.

Résumé français : L'évaluation des effets des contaminants émergents dans les milieux d'eau douce est entravée par le monitorage limité de ces molécules ainsi que par le manque de connaissances sur les effets sublétaux des contaminants sur les organismes aquatiques. Après avoir cartographié divers contaminants dans un bassin versant avec un niveau d'activité agricole intermédiaire, nous avons identifié l'atrazine et le métolachlore comme étant des agents stresseurs d'intérêt parmi les molécules considérés. Nous avons testé les effets de ces herbicides sur des communautés de phytoplancton d'eau douce lors d'expériences individuelles (200 ng L^{-1}). Nous avons évalué une variété de critères d'évaluation, certains utilisés traditionnellement en écotoxicologie (chlorophylle *a*, structure de la communauté d'algues),

d’autres découlant de méthodes développées plus récemment (fluorescence à taux de répétition rapide; séquençage de l'ARN ribosomal 16S; protéomique des isoformes de la protéine de choc thermique). Les traitements au métolachlore étaient caractérisés par une diversité microbienne plus faible, une plus grande abondance relative de protéines de choc thermique et un plus grand nombre d'isoformes exprimées. Les traitements à l'atrazine ont eu moins d'effets, seule une augmentation de l'abondance relative de HSP100 a été observée à cette concentration. Nos résultats suggèrent que les herbicides qui affectent la synthèse des protéines (comme le métolachlore) peuvent induire des réactions de stress dans les communautés de phytoplancton à des concentrations pertinentes pour l'environnement.

Note : À la suite des corrections demandées par les membres du jury, le contenu de cet article diffère de celui qui a été soumis.

3.1 Abstract

Assessment of the effects of contaminants of emerging concern (CECs) in the freshwater environment is hindered by limited monitoring of these molecules and lack of knowledge regarding the sublethal effects of contaminants on biota. Having mapped a variety of contaminants in a watershed with moderate levels of agriculture, we inferred widespread herbicides atrazine and metolachlor to be likely stressors among the molecules considered and tested their effects on freshwater phytoplankton communities in individual experiments (200 ng L^{-1}). We evaluated a number of endpoints, both traditionally used in ecotoxicology (chlorophyll *a*, algal community structure) and more recently developed (fast repetition rate fluorescence; 16S ribosomal RNA sequencing; proteomics of heat shock protein isoforms). Metolachlor treatments were characterized by lower microbial diversity, a greater relative abundance of heat shock proteins and a greater number of isoforms expressed. Atrazine treatments had fewer effects, only an increase in the relative abundance of HSP100 was observed at this concentration. Our results suggest that herbicides affecting protein synthesis (such as metolachlor), can induce stress responses in phytoplankton communities at environmentally relevant concentrations.

3.2 Introduction

The diversity and quantity of synthetic chemicals released in the environment have been increasing at rates greatly surpassing those of other drivers of global change (Bernhardt et al. 2017). Despite this, ecotoxicology remains severely underrepresented in the broader ecological literature (Bernhardt et al. 2017). Assessing the risks of contaminants of emerging concern (CECs) to aquatic food webs poses several challenges, most notably the identification of environmental contaminants and their complex mixtures in the environment and a better understanding of the sublethal effects on a wide range of aquatic organisms (Nilsen et al. 2018).

While great advances have been made in the identification and quantification of CECs, robust data regarding their presence, fate and behaviour in the aquatic environment is lacking (Gavrilescu et al. 2015; Brack et al. 2017). Although molecules can be transported over large

distances (Scheringer 2009), the chemicals present in the environment depend largely upon specific point sources and surrounding land use. Our knowledge of the degree of contamination of the aquatic environment at the scale of the landscape is limited although efforts have been underway to quantify large-scale contamination of ground and drinking water (Lopez et al. 2015; Machado et al. 2016; Torres et al. 2017, Glassmeyer et al. 2017) as well as streams and rivers (Kolpin et al. 2002; Loos et al. 2009; Munoz et al. 2015; Bradley et al. 2017; Elliott et al. 2018). Further efforts have been put towards compiling data from smaller-scale studies (Gavrilescu et al. 2015; Busch et al. 2016). With the exception of very large lakes (Larras et al. 2016, Xie et al. 2015), lakes are generally far less often sampled although recent efforts have led to the quantification of single herbicides (Pollard et al. 2018 ; Castro Berman et al. 2018) and multiple emerging contaminants (Huot et al. 2019) at the scale of the landscape.

Even with robust knowledge of the concentration of contaminants in the freshwater environment, assessing the impact of this contamination on biota presents further difficulties. While there is a large body of research demonstrating the effects of emerging contaminants on aquatic biota, the concentrations at which effects are found have been generally much higher than the concentrations generally detected in the environment (Relyea and Hoverman, 2006). Developed in the 1970s (Truhaut, 1977), ecotoxicology as a field has transitioned from testing differences in species sensitivities to contaminants, to community toxicity testing, and finally to testing real ecological questions in the 1990s (e.g. integrating multiple trophic levels, competition, predation). More recently, tools such as 16S ribosomal RNA (16S rRNA) sequencing have facilitated studying the effect of contaminants on the abundance, diversity and activity of prokaryotic microorganisms (Ghiglione et al. 2016). Unfortunately, beyond the acceptance of community-level tests and the use of functional endpoints (e.g. primary production), environmental standards and regulations continue to be based mainly on single-species tests (Straalen 2003, Gessner & Tlili 2016).

Systems toxicology has been proposed as a method to gain a mechanistic understanding of the ways in which contaminants perturb biological systems (Simmons et al. 2015). Systems toxicology uses enabling technologies to investigate molecular responses to contaminants in order to gain insight on cellular function and organism responses (Sturla et al. 2014). This

information is then used to develop models that allow predicting adverse outcomes at the scale of populations and food webs.

Heat shock proteins (HSPs), some of the best studied proteins, have been long recognized as promising molecular biomarkers in ecotoxicology as their expression is amplified under conditions of stress (see Kregel 2002 for review). Of the families of different molecular weights, the HSP70 are the first HSPs to be induced under stress conditions and are the most evolutionarily conserved HSPs (Gupta et al. 2010). These properties make them attractive biomarkers in ecotoxicology. While HSP gene upregulation can be used as an early molecular endpoint (Morales et al. 2011), proteomics represent another tool to characterize HSP expression. HSP isoforms have been shown to have specific functions (Fernandez-Fernandes et al. 2017; Jacob et al. 2017), and may be differentially expressed during toxicant exposure. In ecotoxicology, bottom-up (shotgun) proteomics are the most commonly used, identifying thousands of proteins without prior knowledge (Martyniuk and Simmons, 2016).

When studying photosynthetic organisms, fast repetition rate fluorescence protocols can provide information on processes occurring within the photosystem II (Kolber et al. 1998; Suggett et al. 2009). This is of particular interest when studying the effect of herbicides, as many of these are inhibitors of photosynthesis (Triazines, triazinones, triazolinone, uracils, pyridazinones, phenyl-carbamates, ureas, amides, nitriles, benzothiadiazine, phenyl-pyridazines, Menne & Köcher 2012).

Through experiments on natural phytoplankton communities, we sought to compare the more traditional eco-toxicological endpoints (chlorophyll *a* concentrations and community structure) with newer tools: 16S ribosomal RNA sequencing (16S rRNA), Fast repetition rate fluorometry (FRRF), and changes in heat shock protein (HSP) isoforms using proteomics.

In the first part, we quantified the number of emerging contaminants in the aquatic environment in a watershed with a moderate proportion of agriculture. This allowed us to identify the molecules most likely to affect phytoplankton communities due to their molecular targets, persistence (detection throughout the year) and concentration. This knowledge informed a series of 2 experiments of moderate length (3 weeks) in which we studied the effects of each individual contaminant at concentrations of the same order of magnitude as those in the environment on a number of endpoints in natural communities that were cultured

in the laboratory. We hypothesized that FRRF and proteomics would be the most sensitive endpoints as they allow tracking the effects of contaminants at the molecular level. We also hypothesized that exposure to contaminants would also result in decreases in microbial diversity as has been shown in the literature (Chakraborty & Bhadury, 2015). The microbial fraction associated to the phytoplankton was likely to impacted to a greater degree than the smaller, unassociated fraction due to indirect effects following effects of the herbicides on the phytoplankton (Marisol et al. 2017).

3.3 Methods

3.3.1 Sampling sites

We quantified the concentrations of a variety of pesticides and pharmaceutical compounds in the Lake Massawippi watershed. This watershed has moderate agricultural activity, and is located in the Eastern Townships, Québec (Canada) (Fig 3.1). Surface water samples were collected in several locations in the two main tributaries of the lake (Rivière Niger, Rivière Tomifobia) using a PTFE dipper or direct surface grabs. Samples from three locations within the lake were collected directly from the surface water into glass amber bottles, kept on ice in the dark, and processed within 24 hours of sampling.

3.3.2 Sample preparation and quantification

Samples were brought to room temperature and filtered through 0.45 µm nylon membrane filters by low-vacuum filtration (5 psi); samples with high sediment loads were prefiltered at 1.2 µm. Duplicate samples of 100 mL were transferred to separatory funnel where 2 g (2% w/v) of NaCl was added and shaken until dissolution of the salt. Ten milliliters of ethyl acetate (10% v/v, Fisher Scientific) were added to the funnels and shaken. After 5 min, the organic phase was collected in a 20 mL vial. The aqueous phase was returned to the funnel and 10 mL of dichloromethane (10% v/v, Fisher Scientific) were added. After shaking and separation of the two phases, the denser organic phase was added to the same 20 mL vial, its contents subsequently evaporated under a flux of nitrogen at 40°C and reconstituted with 1 mL of a 1:1 water methanol solvent (with 0.1% formic acid). Samples were vortexed and sonicated before being filtered by syringe (0.02 µm) into a spectrophotometric vial and frozen

until analysis. Analysis was performed on an Acquity UPLC XEVO TQ mass spectrophotometer (Waters Corporation, Milford, Ma) (see Ba et al. 2014 for details) and the results were processed on Waters Corporation's proprietary software MassLynx.

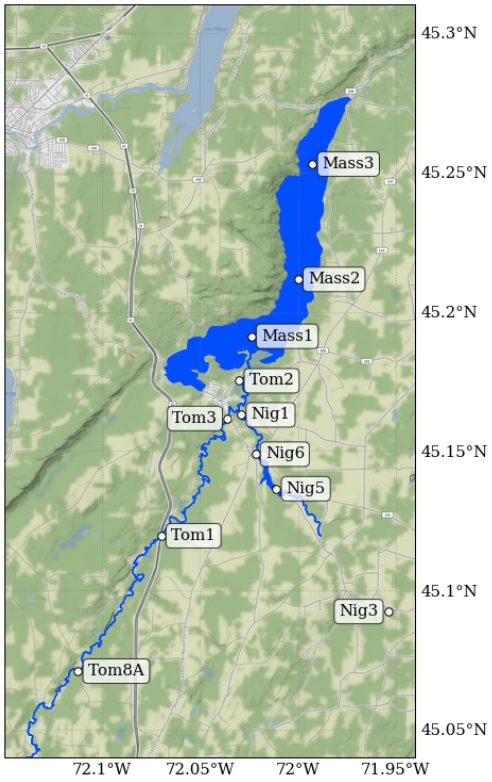

Figure 3.1: Emerging contaminant sampling sites. Green : Forested areas, Beige: Agricultural landscape

3.3.3 Lake culturing experiments

a) Experimental set-up

We assessed the impact on natural phytoplankton communities of the two most often detected herbicides. Lake Massawippi surface water was grab collected and filtered through a 100 µm Nitex screen. Triplicates of the resulting algal communities (herbicide treatment and control) were grown in 4 L glass bottles for 22 days in two separate, sequential, experiments for each molecule tested, following a week of acclimation to laboratory conditions. After acclimation, communities were diluted with modified Bolds Basal media enriched with vitamins (10%) (Andersen 2005) that was replenished throughout the

experiment. We also maintained an additional bottle (abiotic control) containing only the medium and the tested concentration of herbicide, for a total of 7 bottles per experiment.

The first experiment (13-06-2016 to 01-07-2016) was conducted at 18°C and the second experiment (19-08-2016 to 10-09-2016) was conducted at 24°C in order to best mimic the environmental conditions. Bottles were gently stirred using magnetic bars. Light cycles followed a 24h sinusoidal curve of 14 hours light and 10 hours dark with a maximum luminosity of 200 µmol m^{-2} s^{-1}. This luminosity was chosen based on in-situ measurements from lake Montjoie, another lake in the Easter Townships.

b) Daily measurements

Each day, 25 mL was sampled from the cultures to measure photosystem II (PSII) parameters and chlorophyll *a*.

Fast repetition rate fluorometry measurements were taken daily, 30-60 minutes after the initiation of the daily light cycle. From the 25 mL volume sampled in each of the control and treatment bottles triplicates, three volums of 5 mL was transferred into vials and kept in the dark for 20 minutes before being gently mixed by inversion, inserted into the LIFT-FRR fluorometer (Soliense, NY, USA) and measured under a protocol of 100 flashlets over 100 µs followed by a relaxation sequence of 300 µs. This protocol rapidly measures a number of variables of interest: (1) The minimum and maximum fluorescence (F_o and F_m) which are often used as a proxy for algal biomass, (2) the variable fluorescence to maximum fluorescence [$(F_m-F_o)/F_m = F_v/F_m$] ratio, representing the maximum quantum yield of charge separation, which is often interpreted as a measurement of photo-physiological stress, the apparent size of the PSII reaction centers antennae (, nm^2) and (4) the probability of energy transfer between reaction centers (*P*) an estimate of the connectivity of individual reaction centers which changes the rate at which the reaction centers are filled. Finally, the relaxation sequence, allows the calculation (5) the rates of re-oxidation of PSII reaction centers (τ_1, τ_2, τ_3).

Chlorophyll *a* triplicates for each sample (~200 µL) was extracted in 2 mL 3/2 (v/v) acetone 90% - DMSO (MacIntyre and Cullen 2005) and measured using a Trilogy fluorometer (Turner Designs, USA) fitted with a Chlorophyll non-acidification module (Turner Designs, USA).

Pigments were extracted in the dark for 20 min and lightly vortexed prior to fluorescence measurements.

c) Herbicide measurements

The concentrations of atrazine, metolachlor and two atrazine degradation products, atrazine-2-hydroxy and atrazine-desethyl, were measured at five intervals in the cultures during the first experiment and three during the second. The methods were identical to those presented earlier for the environmental samples but with one 100 mL replicate per bottle. We attempted to determine herbicide concentrations associated with the biomass but freeze thawing of the filtered fraction cycles and subsequent solvent extraction did not yield more than trace amounts of herbicides (data not shown).

d) Cell imaging for algal identification, 16S and proteomics

At the end of the experiment samples for cell imagining, 16S ribosomal RNA and proteomics were taken.

Cell imaging by the FlowCAM (Fluid Imaging Technologies, USA) was used to determine the structure of the algal communities at the end of the experiment. We processed 1 mL of sample per bottle and the images were manually classified to determine the relative importance of each group within the community by particle surface area.

For 16S rRNA and proteomics samples, we filtered 500 mL of medium from each experimental unit. We used two 47 mm in-line Filter Holders in serial and a peristaltic pump to collect two size fractions of the bottle communities, >3 µm (3µm filter) and 0.2 - 3 µm (0.2 µm). Both size fractions were used in the 16S rRNA analysis, while only the 3 µm filters were used for the metaproteomics analyses. Samples were conserved in an RNA stabilisation solution and held at -80°C until processing and analysis.

e) Protein and DNA extraction of 3 µm filters

Proteomic and 16S rRNA samples were extracted and using the methods presented in Colatriano and Walsh (2005) which allows the parallel isolation of both proteins and DNA. Briefly, cells were lysed through incubation in SDS extraction solution and a series of centrifugations. The sample volume was split for DNA precipitation (10%) and protein precipitation (90%).

The protein concentrate were precipitated in 4 volumes of methanol:acetone (50:50) to one volume of concentrate, resuspended and quantified using a protein assay kit (Qubit fluorometer; ThermoFisher Scientific, USA). Proteins were extracted using SDS-PAGE gel of proteins, the gel run until the 250 kDa marker just reached the resolving gel. The gel was stained using a coomassie based stain. Each lane was cut into 1mm x 1 mm squares to maximize surface area and pieces were placed in a low-binding centrifuge gel. Samples were de-stained, dehydrated, reduced, alkylated, washed, re-dehydrated and digested before being extracted and resuspended. Peptide solutions were pipetted into nano vials.

The DNA concentrate was extracted with a SDS-extraction solution. Proteins were precipitated with MCP precipitation reagent. The DNA concentrate was precipitated with isopropanol and ethanol and resuspended in low TE buffer. DNA was quantified using a dsDNA assay kit and quality was checked using agarose gel electrophoresis.

f) DNA extraction of 0.2 µm filters

MoBio's PowerWater kit (standard protocol) was used to extract DNA from filters, which was eluted into elution buffer (50 µl). The V4 region of the 16S rRNA gene was PCR-amplified using the U515_F and E786_R standard primers. Conditions were the following: 5 µL Phusion High Fidelity Buffer, 0.5µL dNTPs (10 mM), 1.8 µL U515_F primer (5 µM), 1.8 µL E786_R primer (5 µM), 0.25 µL Phusion polymerase, 13.65µL ddH2O and 2 µL of DNA. PCR conditions were the following: 30 seconds (98˚C), 22 cycles of 20 seconds (98˚C), 35 seconds (54˚C), 30 seconds (72˚C), and a final elongation of 60 seconds (72˚C). Products were pooled and cleaned using the Zymo research DNA purification kit (standard protocol) before elution (30 µL).

In a second PCR reaction, barcodes and Illumina adaptors were added (5 µL High fidelity Phusion buffer, 0.5 µL dNTPs (10 mM), 1.8µL primer PE-PCR-III-F (5 µM), 1.8µL primer PE-PCR-III-XXXX (5 µM), 0.25 µL Phusion polymerase, 11.65 µL dd H2O and 4µL cleaned PCR product). PCR conditions were the following: 30 seconds (98˚C), seven 30 seconds cycles (98˚C), 30 seconds (83˚C) and 30 seconds (72˚C). Samples were then cooled to 10˚C and purified using the AMPure kit (standard protocol replacing 1.8X AMPure XP beads with 0.8X AMPure XP beads). A NanoDrop (ThermoFisher Scientific, USA) was used to measure DNA concentrations. Samples were pooled in volumes of equal DNA quantities and diluted

(10 nM). Samples were sequences using an Illumina MiSeq system (Illumina, USA). Quality control of sequencing plates included two negative controls (ddH2O) and one mock community DNA sample.

g) DNA data processing

Read files were demultiplexed using idemp and Illumina adapters removed with cutadapt. Reads were processed using the DADA2 package (Callahan et al. 2016) in R. Reads were trimmed and merged, chimeras were removed and taxonomy of amplicon sequence variants (ASVs) was assigned to the genus level using the SILVA database v128. From the ASV table obtained, further analysis was conducted using the phyloseq package (McMurdie & Holmes 2013) in R. ASVs from chloroplasts were removed and the data was rarified to a depth of 10 000 reads, leaving a remaining 700 ASVs. Alpha diversity of each sample was calculated using the Shannon index as implemented within the phyloseq package. Significant differences between treatments and controls were tested for through a Monte Carlo permutation test. Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) analysis (Anderson 2014) was used to test for differences in community structure between treatments, experiments and size fractions. The 20 most abundant groups were identified and their relative importance in treatment and control samples were compared.

h) Proteomics analysis and Heat shock protein (HSP) identification

Proteins were identified using the PEAKS software database (see Zhang et al. 2012 for details) by matching the MS/MS spectra of the samples to a custom sequence set, constructed using identified proteins of eukaryotic algae and cyanobacteria obtained through the Joint Genome Institute (JGI) database for all freshwater eukaryotic algae (13 genera), cyanobacteria (26 genera), and freshwater algal communities (1 sample) (Table 3.1). We queried the data for protein sequences identified as being Heat shock proteins, using the terms : “HSP*”, “Heat shock”, “GroES*” , “GroEL*”, ”GrpE*”, ”DnaJ*”, “DnaK*”, “HtpG*”,“Clp*” and “BiP” (the asterisk represents a wildcard). As single peptides sequences were at times matched to multiple protein accessions, we randomly assigned each peptide to a single protein accession. We then calculated the relative abundance of each HSP protein accession by dividing the number of peptide spectras matched to the protein accession by the total number of peptide spectra matched across all proteins in our database.

3.4 Results

Of the 46 molecules (pesticides and other) tested, a number were consistently detected at concentrations greater than their detection limit (Table 3.2). The most abundant molecules detected were herbicides. Atrazine and metolachlor were found at concentrations an order of magnitude greater than the other compounds (Fig 3.2). Concentrations were greatest in the Niger River with a sum of concentrations of all quantified molecules approximating 3 µg L^{-1} while sum of concentrations surpassing 100 ng L^{-1} were rarely observed in Lake Massawippi (Fig 3.3A). Concentration peaks occurred in June and July during periods of strong rains (Fig 3.3B).

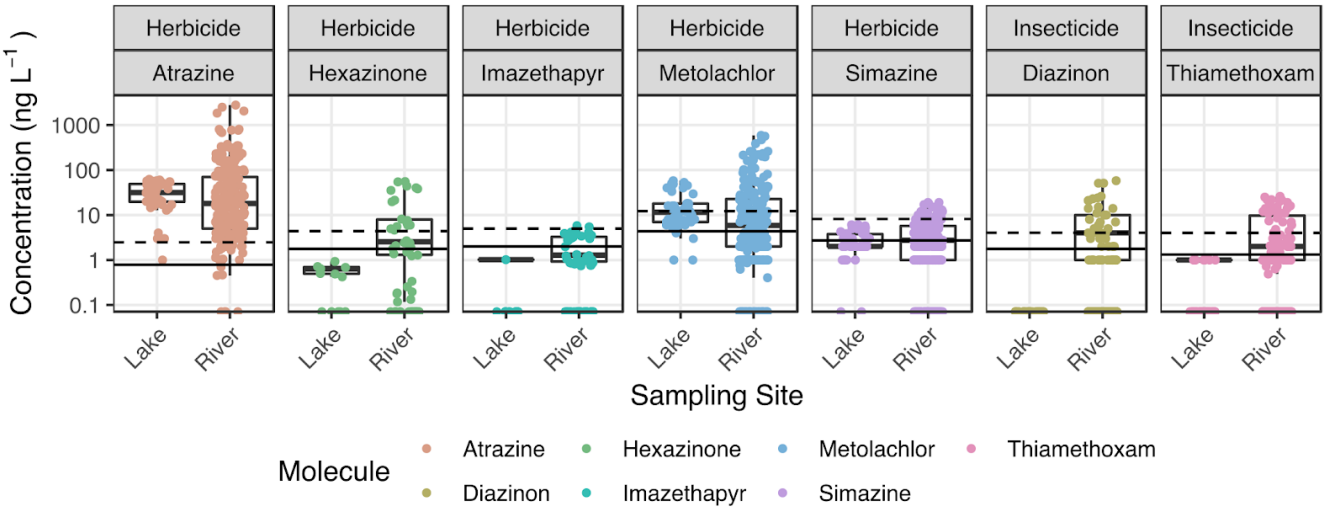

Figure 3.2: Concentrations of the main pesticides detected in Lake Massawippi and its tributaries. Solid line: Detection limit (three times the standard deviation of the blanks); dashed line: Quantification limit (10 times standard deviation of the blanks).

Table 3.1: Protein database for Eukaryotic algae and cyanobacteria (from JGI)

Phylum	**Species/ Genome name**	**Genome ID**
Lake composite	Composite genome from Lake Mendota Epilimnion pan-assembly MEint.metabat.2384	2582580552
Bacillariophyta	*Phaeodactylum tricornutum* CCAP1055/1	649328905
Bacillariophyta	*Thalassiosira pseudonana* CCMP 1335	649328906
Chlorophyta	*Chlamydomonas reinhardtii* CC-503 cw92 mt+	649410502
Chlorophyta	*Chlorella variabilis* NC64A	2507525016
Chlorophyta	*Coccomyxa subellipsoidea* C-169	2614208526
Chlorophyta	*Micromonas pusilla* CCMP 1545	2614208527
Chlorophyta	*Ostreococcus lucimarinus* CCE9901	640281011
Chlorophyta	*Ostreococcus tauri* OTH95	2507525004
Chlorophyta	*Volvox carteri f. nagariensis* 69-1b	2507525017

Cryptophyta	*Guillardia theta*	638269501
Haptophyta	*Emiliania huxleyi* CCMP 1516	2507525023
Ochrophyta	*Aureococcus anophagefferens* CCMP 1984	2507525028
Cyanobacteria	*Anabaena sp*. ATCC 33047	2724679208
Cyanobacteria	*Aphanizomenon flos-aquae* NIES-81	2585427799
Cyanobacteria	*Aphanizomenon flos-aquae* 2012/KM1/D3	2630968589
Cyanobacteria	*Arthrospira maxima* CS-328	642979357
Cyanobacteria	*Calothrix* sp. PCC 7507	2505679032
Cyanobacteria	*Chamaesiphon minutus* PCC 6605	2510436000
Cyanobacteria	*Chroococcidiopsis* sp. PCC 6712	2505679029
Cyanobacteria	*Cyanobium gracile* PCC 6307	2508501011
Cyanobacteria	*Cylindrospermopsis raciborskii* CS-509	2522125118
Cyanobacteria	*Dactylococcopsis salina* PCC 8305	2509276056
Cyanobacteria	*Gloeocapsa* sp. PCC 73106	2508501033
Cyanobacteria	*Halothece* sp. PCC 7418	2503538028
Cyanobacteria	*Hapalosiphon welwitschii* UH strain IC-52-3	2529292566
Cyanobacteria	*Kamptonema formosum* PCC 6407	2508501075
Cyanobacteria	*Leptolyngbya* sp. PCC 6406	2517572073
Cyanobacteria	*Microcystis* sp. PCC 7806SL	2751185885
Cyanobacteria	*Microcystis aeruginosa* NIES-843	641522640
Cyanobacteria	*Microcystis panniformis* FACHB-1757	2645727631
Cyanobacteria	*Nostoc* sp. PCC 7107	2503707008
Cyanobacteria	*Nostoc* sp. PCC 7120	637000199
Cyanobacteria	*Phormidesmis* sp. BC1401	2627853604
Cyanobacteria	*Planktothricoides* sp. SR001 non-axenic culture	2636416084
Cyanobacteria	*Planktothrix* sp. st147	2507262029
Cyanobacteria	*Planktothrix agardhii* NIVA-CYA 34	2506381012
Cyanobacteria	*Planktothrix prolifica* NIVA-CYA 406	2602041638
Cyanobacteria	*Prochlorothrix hollandica* PCC 9006	2509276045
Cyanobacteria	*Pseudanabaena biceps* PCC 7429	2504557005
Cyanobacteria	*Stanieria cyanosphaera* PCC 7437	2503754019
Cyanobacteria	*Synechococcus* sp. PCC 6312	2509276030
Cyanobacteria	*Synechococcus elongatus* PCC 7942	637000308
Cyanobacteria	*Synechococcus leopoliensis* UTEX 625a	2517572104
Cyanobacteria	*Synechocystis* sp. PCC 6803 (updateJune2012)	2514885032
Cyanobacteria	*Tolypothrix* sp. PCC 7601	2501533201

Table 3.2: Molecules analyzed in environmental samples

	Molecule	Detected in samples
Fungicides	Fludioxonil	X
	Boscalid	
	Carbendazim	
	Iprodione	
	Kresoxim Methyl	
	Pyraclostrobin	
	Pyrimethanil	
Herbicides	Atrazine	X
	Hexazinone	X
	Imazethapyr	X
	Metolachlor	X
	Simazine	X
	Linuron	

	Metobromuron	
	Pendimethalin	
Insecticides	Diazinon	X
	Thiamethoxam	X
	Aldicarb-sulfone	
	Azinphos-Methyl	
	Chlofenvinphos	
	Chlorpyrifos	
	Clothianidin	
	Coumaphos	
	Dimethoate	
	Imidacloprid	
	Malathion	
	Nitenpyram	
	Parathion	
	Permethrin	
	Phosmet	
	Thiacloprid	
Pharmaceuticals	Acetaminophen	X
	Caffeine	X
	Carbamazepine	X
	Cyclophosphamide	X
	Fenofibrate	X
	Ifosfamide	X
	Indomethacin	X
	Mefenamic Acid	X
	Ofloxacin	X
	Trimethoprim	X
	Atenolol	
	Ciprofloxacin	
	Ibuprofen	
	Ketoprofen	
	Naproxen	

We focused our efforts on testing the effects of the two predominantly detected contaminants atrazine and metolachlor at target concentrations of 200 ng L^{-1} This value is three times that of the maximal sum of herbicide concentrations observed in the lake. While these concentrations exceeds those found in lake Massawappi, they are inferior to concentrations often found in the lake's tributaries and were deemed likely to be encountered in smaller, more contaminated systems.

3.4.1 Herbicide concentrations during the experiment

Although the target concentration applied was 200 ngL^{-1}, herbicide concentrations measured in the microcosms during both experiments varied between 50 and 200 ng L^{-1} (Fig 3.4) (note:

although inferior to the target concentration, possibly due in part to adsorption of the molecules to the glass containers [Sharom & Solomon 1981], these remain greater than concentrations measured in the lake). For the atrazine treatment, the decrease of deethyl-atrazine, a degradation product likely originating from the initial lake water, was observed in the treatment microcosm (Fig 3.4A). Atrazine-2-hydroxy, another degradation product was observed to increase both in the treatment and the abiotic control bottles (Fig 3.4A).

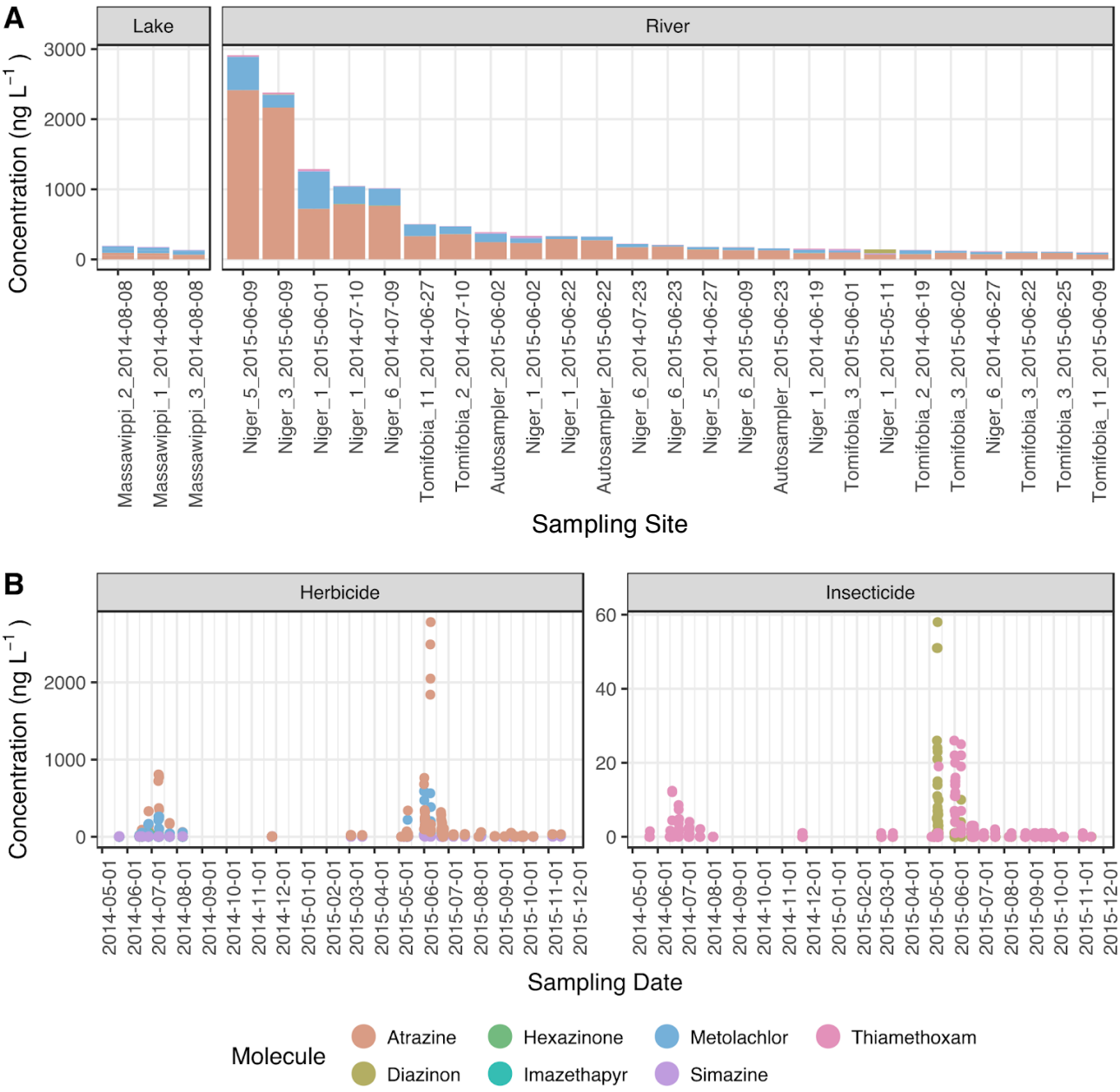

Figure 3.3: Maximum pesticide concentrations found in the environment. A) Total pesticide concentrations at individual sampling points (cutoff value set at 100 ng L^{-1}). B) Seasonal pesticide concentrations in lake Massawippi's tributaries (samples from single location where autosampler was located).

3.4.2 Extracted chlorophyll *a* and FRRF

Although replenishment volumes varied between days, the mean dilution rate was 0.1 day^{-1}. Growth rates and biomass as estimated by chlorophyll *a* did not vary between treatments and controls for either experiments (Fig 3.5A). The same was found for photophysiology

endpoints (Fig 3.5B). The F_v/F_m ratio was variable during the atrazine experiment and P and τ_1 increased over time. For the metolachlor experiment, P decreased over time. While the values of most measurements were very consistent between bottles within experiments, Control a (C_a) in the metolachlor experiment was an exception. For unknown reasons, chlorophyll *a* concentrations in this bottle was double that of the other microcosms in the last days of the experiment. It also yielded greater F_v/F_m, P and τ_1 values than the other microcosms.

By the end of the experiments, FlowCam imaging showed no significant differences in community structure between controls and treatments (Fig 3.6). Communities in both experiments were dominated by *Scenedesmus Opoliensis*. Other scenedesmales were also present (*S. Longispira*, *Actinadestrum* sp. and *Ankistrodesmus* sp.). Likely due to seasonal sucession, the metolachlor experiment had a greater number of diatom species both in control and treatment, while a greater number of small solitary cells were observed during the atrazine experiment. Further analysis of the image properties themselves did not reveal any notable differences regarding particle properties between treatment and controls (diameters, area, aspect ratio, transparency, intensity; data not shown).

3.4.3 16 rRNA

PERMANOVA analysis did not show any significant effect of herbicide treatment on microbial communities. It did, however, find significant differences in the community structure between the two size fractions considered (R^2=0.19; F=5.82; p-value=0.002) and between experiments (R^2= 0.09; F=2.75; p-value = 0.033). This is unsurprising as free-floating bacterial and those associated with algae are likely to have different ecologies, both affected by seasonal succession. Shannon diversities of the two size fractions were not significantly different in the atrazine experiment, but for metolachlor, average Shannon diversity of the 0.2 µm fraction decreased significantly from 3.31 in the control to 2.62 in the treatment (Monte Carlo exact permutation test (MCEPT), p-value=0.047, Fig 3.7). There was no difference between treatments and controls in the 3 µm fraction suggesting that the bacterial community associated to the algae was less affected than the free floating bacteria.

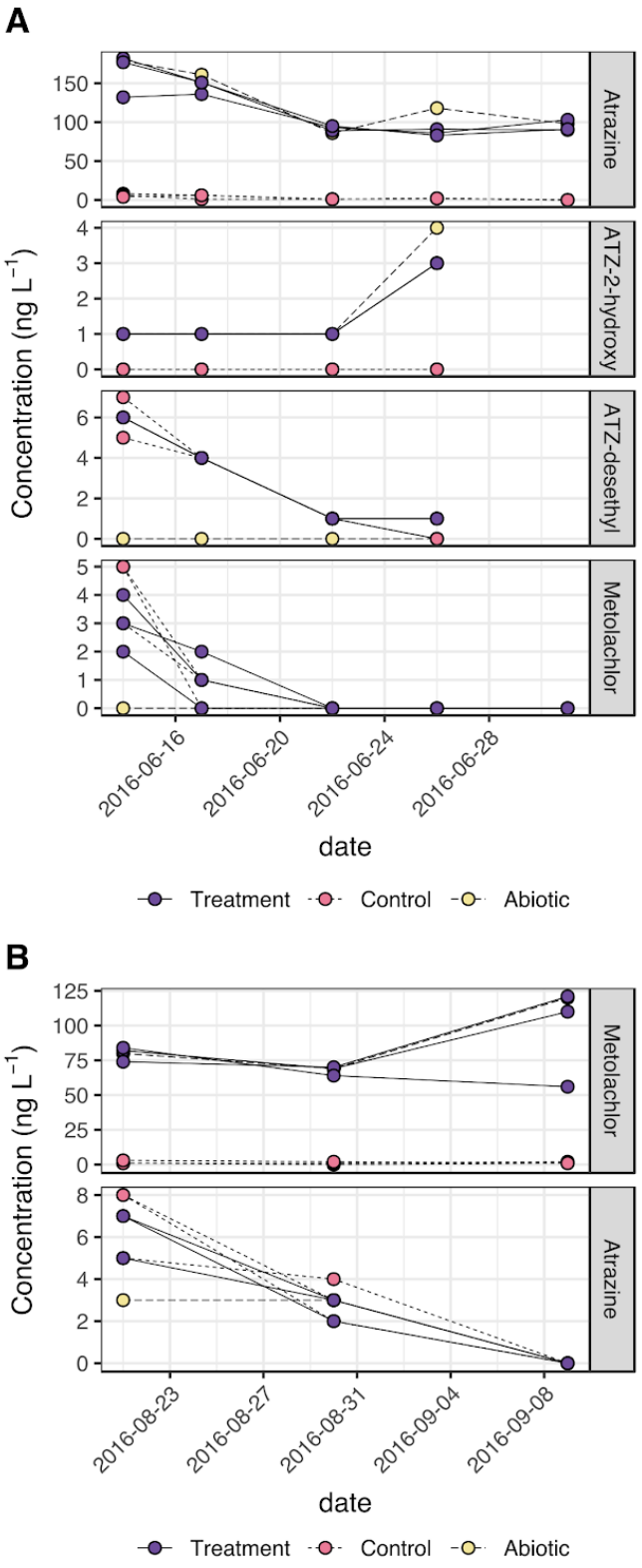

Figure 3.4: Concentrations of herbicides and transformation products concentrations during the experiments. A) Atrazine experiment, B) Metolachlor experiment

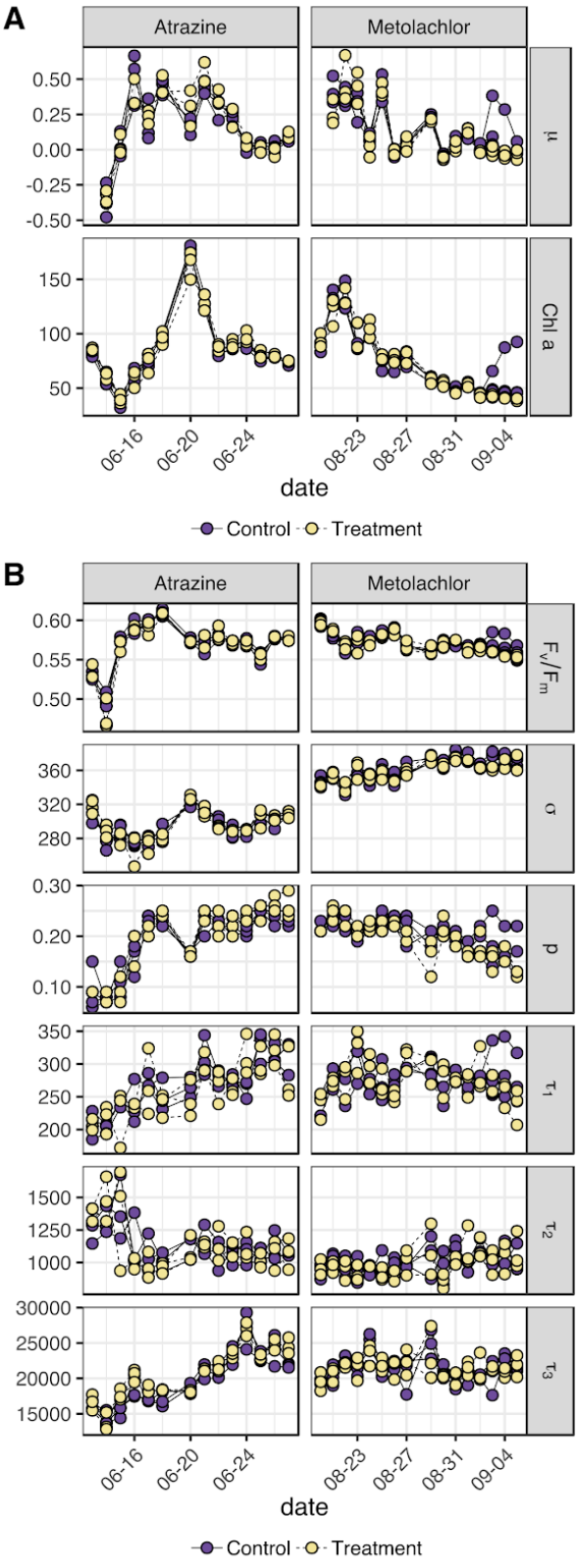

Figure 3.5: Daily measurement of A) extracted chlorophyll *a* and growth rates, B) Selected FRRF parameters

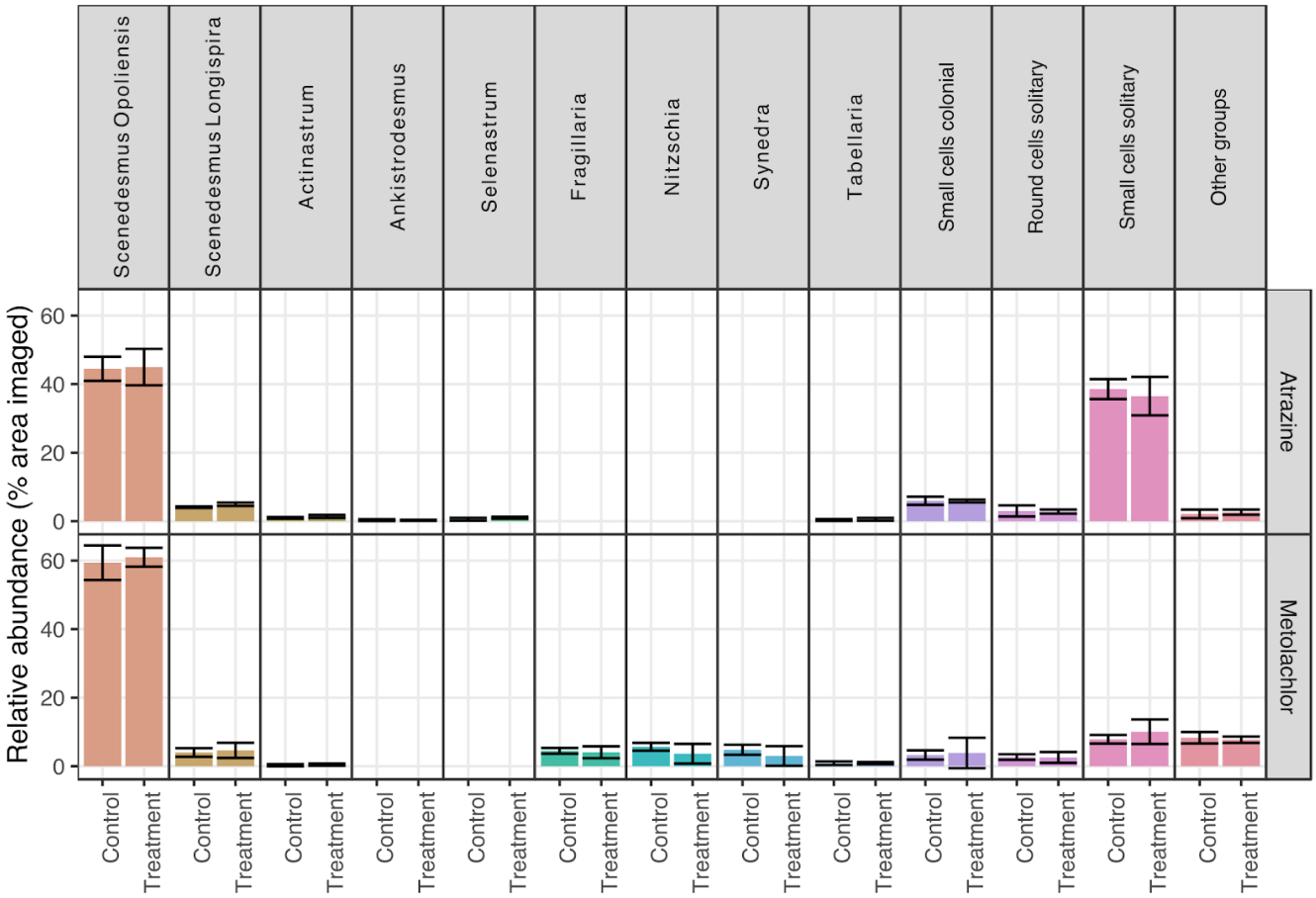

Figure 3.6: Algal community structure for both experiments as determined by FlowCAM imaging and manual sorting

When considering the 20 most abundant taxons based on ASV, we observed few changes between treatment and controls (Fig 3.8). In the 0.2 fractions, the only notable changes were the apparent decrease in the abundance of *Flavobacterium* and *Aquabacterium* in the metolachlor treatment. The database did not resolve all ASVs to the genus level Consideration of higher taxonomic levels did not yield further observable trends (data not shown). When comparing between experiments, the most abundant genera in the 0.2 µm fractions were generally conserved between experiments while more notable changes were observed between the 3 µm fractions in both experiments, with *Pseudoanabaena* and *Reyranella* being exclusive to the atrazine experiment, as was *Sediminibacterium* to the metolachlor experiment.

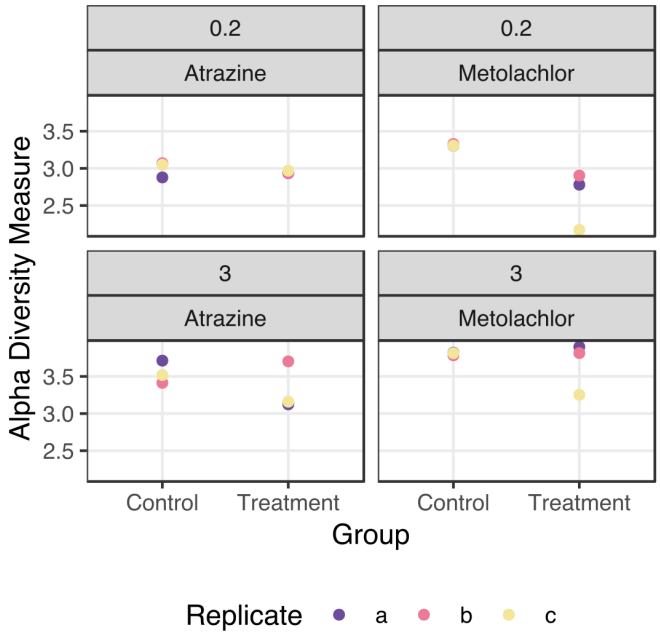

Figure 3.7: 16S Shannon diversity index for the 0.2 µm (top) and 3 µm (bottom) size fractions.

3.4.4 Protein analysis

Query of the protein output revealed 247 unique HSP protein accessions. Within the samples, 682 matches were made. Of these, 343 were from cyanobacteria and 268 were from Chlorophyta with the remaining 71 split between other samples (natural lake communities> Choanozoa > Bacillariophyta> Haptophyta> Cryptophyta). Cyanobacterial HSPs were the most abundant HSPs in the atrazine experiment, while eukaryotic HSPs were the most abundant in the metolachlor experiment (Fig 3.9A). Differences between controls and treatments were found between the relative abundance of HSPs during the metolachlor experiments (Fig 3.9A). The relative abundance of all HSPs was significantly greater in microcosms treated with metolachlor (Control: 4.76% ±1.03%, Treatment: 6.97%±0.68%; MCEPT p=0.047). Among HSP groups considered (HSP60 [GroEL], BiP, HSP70, HSP90, HSP100[Clp]), HSP100 were more abundant in the treatments than in the controls (Control: 0.62% ±0.16%, Treatment: 1.20%±0.12%; MCEPT p=0.047). Interestingly, this was also the case for atrazine treatments, although this was the only effect observed (Control: 0.97% ±0.16%, Treatment: 1.45%±0.33%; MCEPT p=0.047). HSP70 were marginally more abundant in the metolachlor treatment bottles relative to the control (P=0.1)(Fig 3.9B). The lack of observed effects of metolachlor on HSP70, HSP90 and the smaller 20-40 kDa HSPs

was likely due to higher relative abundances in the C_a bottle, which were comparable to those found in the treatment bottles. The C_a bottle was the one in which we observed chlorophyll *a* concentrations double that of the other microcosms. As no other noticeable differences were observed between bottles, we consider it likely that this difference in cell density was sufficient to elicit a HSP response, possibly due to the fact that HSPs are also required in normal cellular processes or because the conditions under greater biomass induced a stress response comparable to the one elicited by low concentrations of metolachlor.

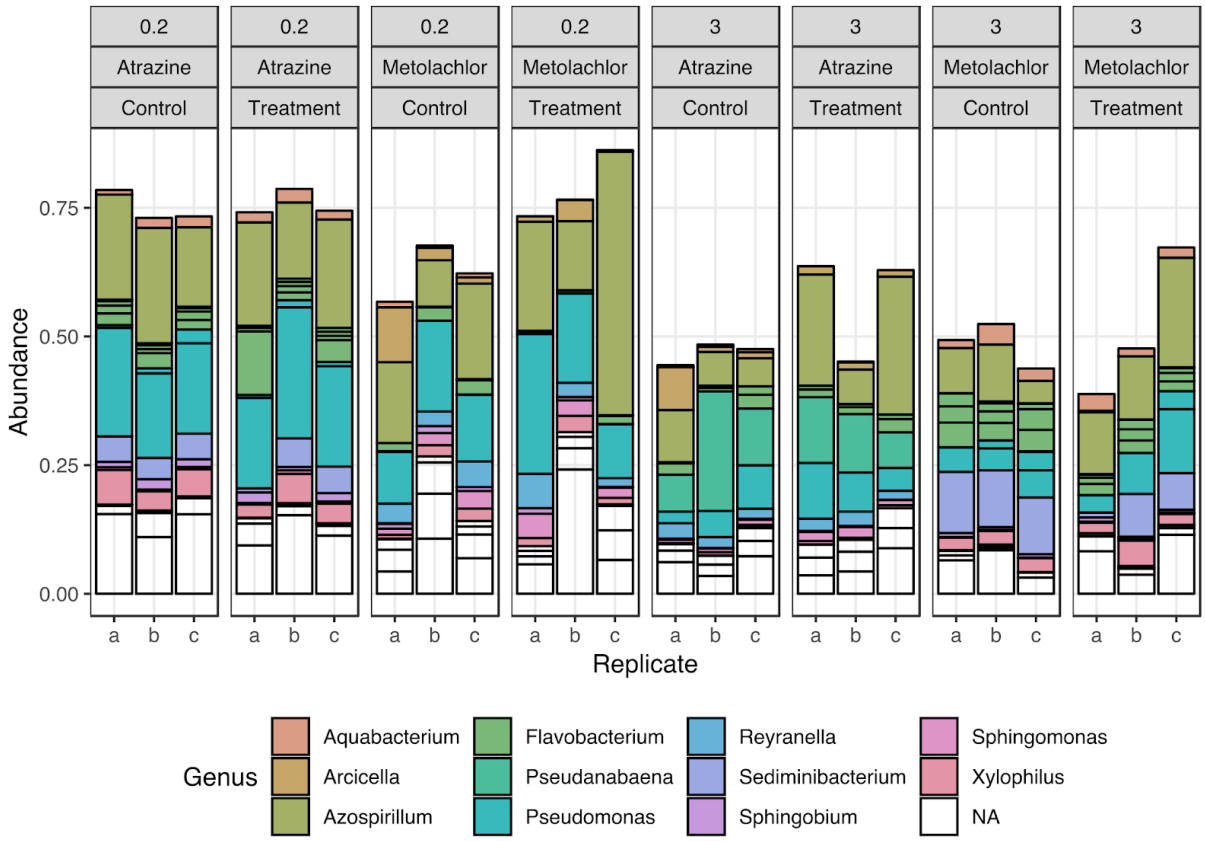

Figure 3.8: Top 20 bacterial genera identified in the samples. Empty boxes (NA) represent OTUs not identified to this taxonomic level. Numbers at the top of each panel represent the filter pore size.

Metolachlor treatments were also associated with an increase in the number of various HSP70 and HSP90 isoforms (Fig 3.10). We note however that different isoforms were found within replicate samples. This may suggest that sequence overlap with that of the database were insufficient to consider the data at this resolution. Protein isomers were near-exclusively

matched from proteins from 4 eukaryotic species (*Chlamydomonas*, *Micromonas*, *Ostreococcus* and *Volvox*). While fewer differences were observed in the atrazine treatments, we note that while HSP70 specific to chloroplasts was found in one control microcosm, it was expressed in all treatment microcosms (Data not shown).

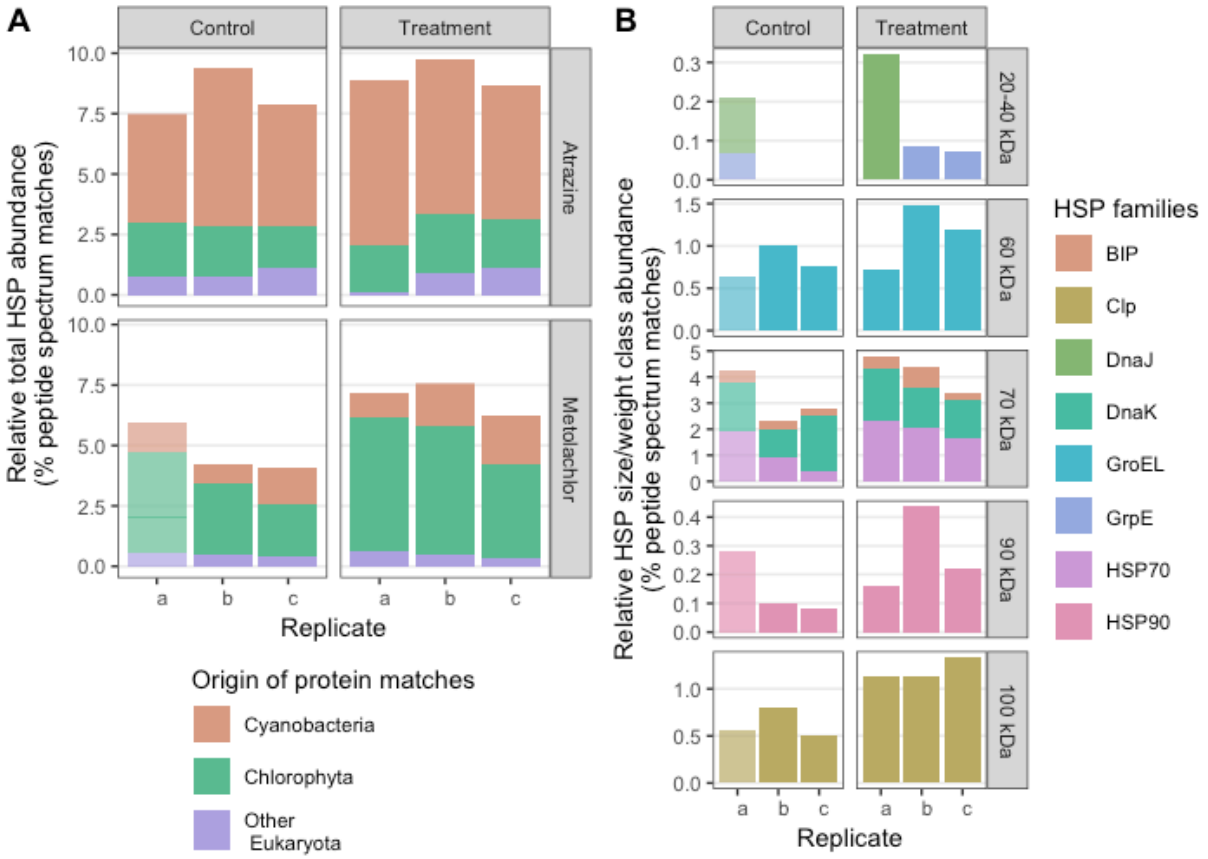

Figure 3.9: Relative abundance of HSPs. A) Relative abundances of HSPs from both experiments. B) Relative abundance of HSPs from metolachlor experiment according to HSP size class and protein family. Shown with transparency is the metolachlor control C, that differed from other microcosms in the experiment by significantly higher biomass (as determined by chlorophyll *a* concentration)

3.5 Discussion

We sought, through this work, to assess the effect of environmentally relevant concentrations of contaminants of emerging interest on phytoplankton communities. The inclusion of molecular measurements in the endpoints considered allowed to observe physiological

changes at very low contaminant concentrations, which are likely to be encountered in various freshwater ecosystems.

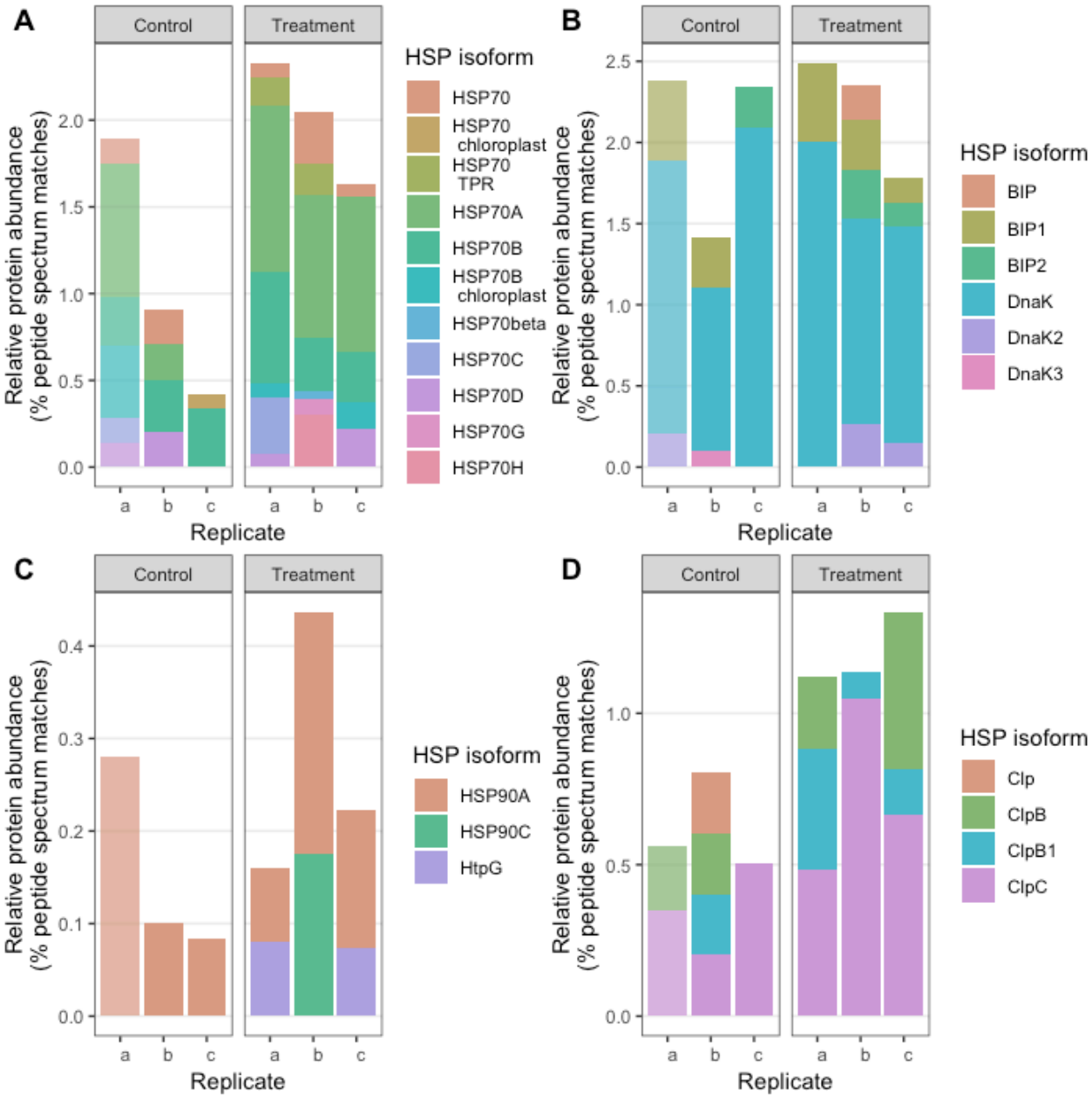

Figure 3.10: Relative abundances of isoforms from larger (>60 kDa) HSPs. A) Eukaryotic HSP70, B) Prokaryotic HSP DnaK and Eukaryotic HSP70 BiP [Endoplasmic reticulum]), C) HSP90 family, D) HSP100 family

The contaminant concentrations we found in the environments, which informed the molecules and concentrations used in the experiments, are in line with the results from the Québec

government's monitoring of Québec rivers (Giroux, 2015). While lake samples were underrepresented in our sampling scheme relative to river sampling, other research has demonstrated that, based on the list of molecules of Table 2, atrazine, metolachlor, imazethapyr, caffeine, fludioxinil and trimethoprim were the molecules consistently detected in the lake at concentrations greater than the detection limit (Simone Kapusta, pers. comm.).

While herbicides at concentrations found in the environment may cause no changes in commonly employed phytoplankton metrics, such as chlorophyll *a* concentrations and community structure, changes in microbial communities and phytoplankton physiology may still occur. Metolachlor induced the surexpression of heat shock proteins and increases in the number of HSP70 and HSP90 isomers expressed. While no effects were observed on the bacterial communities associated with the bulk of the phytoplankton, likely because this trophic level was mostly unaffected, the small 0.2 µm bacterial fraction saw a decrease in community diversity. Comparable concentrations of atrazine yielded fewer changes in the metrics considered. Atrazine treatment resulted in a greater relative abundance of HSP100 proteins. More strikingly, the relative abundance of HSP100 doubled on average in the treatments of the metolachlor experiments. Differences were not found to be associated with differences in the relative abundance of the cyanobacterial and eukaryotic proteins matched in the experiments and may be due to differences in the mode of action of the two herbicides, as metolachlor targets protein synthesis and might induce a greater need for protein refolding.

The primary site of action of metolachlor and other chloroacetanilides is the FAE-synthase, a starter enzyme necessary for the elongation of C16 to C20 fatty acids (Götz and Böger, 2004). Through the inhibition of cell division and cell enlargement, the molecule halts seedling shoot and root development of target plants (Deal and Hess, 1980). In algae this has been found to affect cell rigidity and permeability (Schmalfuss et al. 1998; Böger, 2003). It has been shown that alachlor, another chloroacetanilide, induces oxidative stress due to protein denaturation and an upregulation of genes encoding for proteins in the HSP70 and HSP90 families (Rattanawong et al. 2015, reviewed in Moreira-de-Sounsa et al. 2018). Although somewhat masked by changes in the highly productive C_a bottle, our results provide further support for the importance of these proteins in managing chloroacetanilide stress at sublethal concentrations. Furthermore, we provide further evidence of the increase of the HSP100 (Clp)

under metolachlor exposition. The HSP100 could prove to be a better biomarker of herbicide stress as, in this study, they appear comparatively unaffected by effects other than the herbicide. While heat shock proteins play a key role in mediating stress, they are also increasingly recognized for their role in plant proteostasis control and growth (Hartl et al. 2011, Wang et al. 2015) and their surexpression in the C_a bottle undergoing excessive growth may reflect this key role. It is understood that HSP100 (Clp) are responsible for protein disaggregation through cooperation with HSP70 (Mishra and Grover 2015, Mogk et al. 2015) and thus may be more specific to stress tolerance than HSP70 and HSP90. While a co-occuring focus on the expression patterns of the genes coding for HSPs (transcriptomics) could provide further confirmation of the upregulation of their production, the shot-gun proteomics approach used here proves useful for semi-quantitative analysis of HSPs, and furthermore can provide novel information regarding the functional differences in HSP isoforms.

Organisms generally possess a variety of HSP70 isoforms but little is known of their respective functional relevance. It has, however, been shown that they may differ in expression under stress and, when mediating cellular stress, present functional differences (Waller et al. 2018). This has also been shown for HSP90 (Prince et al. 2015). The HSP70 family is the most evolutionarily conserved and best-studied class of HSPs (Lanneau et al. 2008). In our communities this family had the greatest number of resolved matches in the database. This may explain why it was the family for which we observed the greatest changes in the abundance and number of isoforms identified, if results from the C_a bottle are omitted. Relatively few omics studies have been performed on freshwater phytoplankton (Jamers et al. 2009), consequently the protein database against which our results were matched consisted of relatively few species. For example, the matches for HSP70 proteins came from 6 species, of which 5 were of the Chlorophyta phylum. While we demonstrate that proteomics of HSPs can be used to detect significant changes in the stress response of natural communities, increasing the database of model organisms proteomes will likely further improve the use of this technique for comparative metaproteomics of freshwater phytoplankton communities.

The effects of metolachlor on HSP isomer expression appear to be mostly limited to HSP matched to eukaryotic algae. While cyanobacterial HSP70s were matched within 19 species,

no differences were observed in the isoform response of the cyanobacteria although it has been shown that specific isoforms of the HSP70 prokaryotic DnaK protein are up-regulated under various stress conditions (Rupprecht et al. 2010). Cyanobacterial HSPs represented a smaller fraction of the community compared to the atrazine experiment. Comparing the proteomic response of communities where cyanobacteria are dominant could address whether these organisms are more tolerant to metolachlor stress.

Our results suggest that metolachlor has a deleterious effect on bacterial community diversity, consistent with previous studies (Pesce et al. 2009; Aguayo et al. 2013; Muturi et al. 2017). *Flavobacteria* (Bacteriodetes) and *Aquabacterium* (Proteobateria) are widely distributed in the environment, where they are associated with the degradation of complex organic compounds (Bissett et al. 2008; Kolton et al. 2016; Wilson et al. 2016; Jiao et al. 2017). While their apparent sensitivity to metolachlor in the 0.2 μm fraction of the biomass is somewhat surprising, they remain among the important genera represented in the fraction of the microbial community associated with the algae.

The increase in hydroxyatrazine in atrazine treatment microcosms suggests that the degradation of atrazine occurs through hydrolysis, and the formation of this intermediate dehalogenated product (Shapir et al. 2007). However the concomitant increase of hydroxyatrazine in our abiotic control, prepared under sterile conditions, suggests that the formation of this degradation product is not only of biotic origin. It has been demonstrated that atrazine biodegradation has a lower limit of ~10 μg L^{-1} which could explain the lack of observed biodegradation in our microcosms (Kundu et al. 2019).

Although single herbicide treatments demonstrated effects on microcosm communities, combinations of low concentrations of many contaminants (“cocktail” of contaminants) may, however, represent a far greater risk as the sum concentration may be significantly higher, with possible direct and indirect effects (Relyea 2009). Furthermore, as phytoplankton communities undergo seasonal succession, it is important to consider how seasonality can affect the impact of herbicides on natural communities (Bérard et al. 1999; Pesce et al. 2009; Larras et al. 2014). While the differences in the response of communities to atrazine and

metolachlor is likely due to the specific mode of action of the latter, changes in community structure between experiments may also play a role.

3.6 Conclusion

We demonstrate the effects of low concentrations of metolachlor on the relative abundance of HSP, the expression of HSP isoforms and bacterial community structure. In contrast, atrazine treatments were only associated with increases in the relative abundance of the larger HSP100. These changes occurred without impacting the chlorophyll *a* concentration of phytoplankton communities or their structure, two of the more commonly considered endpoints in ecotoxicological studies of phytoplankton communities. While decreases in bacterial community diversity due to pollution may represent a risk of decreased resilience to other environmental changes (Garcia-Armisen et al. 2014), the consequences of changes in HSP expression without any concomitant structural or functional effects at the community are much more difficult to assess. The HSP molecular chaperones protecting organisms from a number of environmental stressors are encoded by highly conserved genes, which are expressed in every species studied (Feder and Hoffman, 1999). Metolachlor exposure at low concentrations induces a physiological response that appears to maintain the functional and structural integrity of phytoplankton communities, albeit at the likely cost of energy expenditure. As omics become more abundantly used in the study of environmental contaminants, better definitions of what qualifies as harm may be required. While community structure studies are suitable to assess the ecological quality of the aquatic environment, molecular biomarkers, such as HSPs, may serve as early-warning systems and investigate the causes of ecological impairments (Martinez-Haro et al. 2015). As it becomes increasingly clear that aquatic contaminants can change populations through multi-generation exposure (Médina et al. 2007, Oziolor et al. 2016), acclimative physiological responses may ultimately prove important to overall ecosystem functioning.

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CHAPITRE 4

INHIBITEURS DU PHOTOSYSTÈME II ET FLUORESCENCE À TAUX DE RÉPÉTITION RAPIDE

Avant-propos

Auteurs et affiliation :

M. Beaulieu : étudiante au doctorat, Université de Sherbrooke, Faculté de génie, Département de génie civil.

H. Cabana : professeur, Université de Sherbrooke, Faculté de génie, Département de génie civil

Y. Huot : professeur, Université de Sherbrooke, Faculté des lettres et sciences humaines, Département de géomatique appliquée

Date de soumission : 20 août 2019

Revue : Science of the Total Environment (Accepted with major revisions)

Titre anglais : Adverse effects of atrazine on phytoplankton cultures and communities at environmentally relevant concentrations observed using Fast Repetition Rate Fluorescence

Titre français : Effets néfastes de l'atrazine sur les cultures et les communautés de phytoplancton à des concentrations retrouvées dans l'environnement observées grâce à la fluorescence à taux de répétition rapide

Contribution au document : Cet article contribue à la thèse en démontrant que l'atrazine et le DCMU, des inhibiteurs du photosystème II, inhibent la photo-physiologie du phytoplancton à des concentrations plus faibles qu'antérieurement démontré. Cette méthode sensible et facile à utiliser ne démontrait pas d'effets dans la sensibilité des cyanobactéries et microalgues eucaryotes.

Résumé français : La contamination généralisée et persistante des milieux d'eau douce par les faibles concentrations de contaminants émergents est une préoccupation croissante dans le monde. En milieu aquatique, la pollution par les herbicides représente un risque particulier pour le phytoplancton, en raison de leurs similitudes avec les plantes terrestres. En utilisant la fluorométrie à taux de répétition rapide (FRRF) au cours d'expériences d'une semaine sur 10 cultures de phytoplancton appartenant à 4 classes et 4 communautés naturelles, nous avons démontré que les herbicides inhibant la PSII, notamment l'atrazine, largement utilisé en Amérique du Nord, ont des effets constants sur la photophysiologie du phytoplancton d'eau douce à des concentrations bien inférieures aux concentrations affectant les espèces les plus sensibles dans des études antérieures. Les paramètres spécifiques à FRRF ($P, \sigma, \tau_1, \tau_2, \tau_3$) étaient ceux qui étaient les plus sensibles aux inhibiteurs de PSII, comparés aux paramètres de fluorescence standard dérivés d'autres protocoles de fluorescence tels que la fluorométrie à modulation d'amplitude d'impulsion (PAM) (F_0, F_m, F_v/F_m) et aux concentrations de chlorophylle *a*. Selon ces observations, les directives et normes nationales en vigueur concernant l'environnement ne semblent pas suffisantes pour prévenir de manière adéquate les effets négatifs de l'atrazine et d'autres herbicides inhibiteurs de PSII sur les écosystèmes aquatiques et devraient être réévaluées.

Note : À la suite des corrections demandées par les membres du jury, le contenu de cet article diffère de celui qui a été soumis.

4.1 Abstract

The widespread and persistent contamination of freshwater environments by low concentrations of trace organic contaminants (TROCs) is a growing concern worldwide. In aquatic environments, herbicide pollution is of greatest concern for phytoplankton, due to their similarities to terrestrial plants. Through the use of Fast Repetition Rate Fluorometry (FRRF) during weeklong experiments on 10 phytoplankton cultures from 4 classes and 4 natural communities, we demonstrate that PSII-inhibiting herbicides, notably atrazine that is extensively used in North America, consistently have effects on freshwater phytoplankton photophysiology at concentrations far below concentrations affecting the most sensitive species in previous studies. The parameters specific to FRRF ($P, \sigma, \tau_1, \tau_2, \tau_3$) were those most sensitive to PSII inhibitors, compared to the standard fluorescence parameters derived from other fluorescence protocols such as Pulse Amplitude Modulation (PAM) fluorometry (F_0, F_m, F_v/F_m) and extracted chlorophyll *a* concentrations. Based on these findings, existing national environmental guidelines and standards may be insufficient to adequately prevent adverse environmental effects of atrazine and other PSII inhibiting herbicides in aquatic ecosystems and should be re-evaluated.

4.2 Introduction

Weed management strategies in North America have relied heavily on chemical inputs for the past half-century. Reliance on hand pulling and crop rotation was quickly replaced with herbicide use for weed management, as their use was found to be efficient, low-cost and far less labour-intensive (Kraehmer et al., 2014). Consequently, herbicides are amongst the most frequently detected trace organic contaminants (TROCs) in the environment, sometimes decades after being banned, as in the case of atrazine in German groundwater despite its 1991 ban (Vonberg et al., 2014, Gavrilescu et al., 2015). Inland surface waters are particularly vulnerable environments (Dudgeon et al., 2006) and those in proximity to agricultural lands are likely to be contaminated by multiple herbicides (Malaj et al., 2014, Gavrilescu et al., 2015).

In freshwater environments, likely due to their similarities to land plants, phytoplankton and macrophytes have been found to be the most susceptible to harmful effects from many herbicides (Rochon et al., 1999, Malaj et al., 2014). Land plants evolved between 360 and 480 million years ago from a common algal ancestor (Kenrick and Crane, 1997). The core photosynthetic electron-transport pathways have remained highly conserved in higher plants, eukaryotic algae and cyanobacteria, with the exception of peripheral components. Notably, the phycobiliosomes of cyanobacteria have been replaced with membrane intrinsic light-harvesting complexes in eukaryotes (Allen et al., 2011). This makes photosystem-inhibiting herbicides a considerable concern to phytoplankton.

The fluorescent properties of chlorophyll *a* *in-vivo* are a potentially powerful tool to investigate the effects of photosystem II (PSII) inhibitors on natural phytoplankton communities. Despite the crucial role of herbicides in elucidating the photosynthetic light reactions, relatively few studies have in turn exploited *in-vivo* fluorescence protocols to study the impact of herbicide use on the reactions of aquatic algal communities (Séguin et al., 2002, Guasch et al., 2003, Juneau et al., 2007, Knauer et al., 2010, Knauer et Hommen, 2012, Smedbol et al., 2018). Such protocols are relevant given that many commonly used herbicides target the photosynthetic pathways of photosynthetic organisms. Of these, most of these photosynthesis inhibitors impact the photosystem II (Cobb and Reade, 2011) where most of the *in-vivo* chlorophyll *a* fluorescence originates. The most notable of these is undoubtedly atrazine, the second most widely used herbicide in the United States (Atwood and Paisley-Jones 2017). Although its use in Canada is far less important, atrazine remains one of the most frequently detected herbicides in surface waters in provinces where corn cultivation is important (Giroux, 2015). Due to its persistence and toxicity (Singh et al., 2018), atrazine is a molecule of great interest and concern.

4.2.1 Extracted and *in-vivo* chlorophyll *a*

The fluorescence emission of extracted chlorophyll *a* is routinely used as a proxy for phytoplankton biomass. This method yields comparable results to that of spectrometric analysis of chlorophyll *a* (Gregor and MarŠálek 2004). While it retains the same assumption that the chlorophyll *a*: biomass ratio is constant between species and across environmental gradients, it is a far more rapid and sensitive method. When measured *in-viv*o, chlorophyll *a*

fluorescence provides, in addition to a proxy of biomass, a suite of information relating to the photophysiology of photosystem II, most notably the quantum yield of charge separation (Φ_{PSII}), which can be used as a measure of algal stress (e.g., Parkhill et al., 2001 but see Suggett et al., 2009). In addition to being more rapid than extracted chlorophyll *a* methods, the measurements taken through *in-vivo* fluorescence protocols are non-destructive, which allows tracking changes in an experimental unit through time. These characteristics should be of great interest to those conducting ecotoxicological experiments as balancing sufficient treatment levels, experimental power and time and space constraints can be challenging. It is important to consider that there exists multiple situations where the concentration of photosynthetic pigments do not correlate with biomass, notably in the presence of contaminants, where the generation of reactive oxygen species can degrade pigments.

4.2.2 *In-vivo* fluorescence protocols

Pulse amplitude modulation (PAM) fluorometry and fast repetition-rate fluorometry (FRRF) are commonly used protocols for in vivo fluorometry. While a number of studies focus on rapid rise chlorophyll *a* fluorescence induction and the Pulse-Amplitude-Modulated (PAM) fluorometry (Séguin et al., 2002, Guasch et al., 2003, Juneau et al., 2007, Knauer et al., 2010, Knauer et Hommen, 2012, Magnusson et al., 2012, Deblois et al., 2013), the use of the fast repetition rate fluorescence (FRRF) protocol has, to our knowledge, not been used to study the toxicological effects of herbicides on natural phytoplankton communities. In contrast to standard PAM protocols, FRRF does not induce the relaxation of quenching as is seen in PAMs multiple turnover protocol (Kromkamp and Forster 2003). This observed rise in fluorescence is thought to be the result of reduction of the secondary electron acceptor QB and the reduction of the plastoquinone pool (PQ). The FRRF protocol also has the advantage of being more sensitive than standard PAM protocols and allows additional parameters to be derived on the PSII antenna size, and connectivity of the PSII pool (Kolber et al., 1997, Suggett et al., 2003).

We used FRRF to study the effects of three herbicides on diverse phytoplankton cultures and phytoplankton communities. Atrazine and metolachlor were chosen for being widely used for crop protection, persistent and moderately water-soluble. The former is a PSII inhibitor while the latter targets protein synthesis. Both molecules are used in corn production and are

routinely detected in the aquatic environment. In streams adjacent to fields in Québec (Canada), detection rates are near 100% throughout the year with maximum concentrations of around 10 $\mu g\ L^{-1}$ for both molecules (Giroux 2015). In Ontario (Canada), when stream sampling was not limited to agricultural areas, molecules remained detected in all areas tested, with average concentrations of 0.17 $\mu g\ L^{-1}$ atrazine and 0.10 $\mu g\ L^{-1}$ metolachlor found (Byer et al., 2011). Detection of herbicides and their concentrations when present appears to be generally lower in lakes, for example atrazine is detected in 30% of US lakes at with an average concentrations of 0.12 $\mu g\ L^{-1}$ (USEPA 2016). In addition to their ubiquity in the environment, the ecotoxicological risk of these molecules has been relatively well studied (Séguin et al., 2001, Hayes et al., 2010, Dorigo et al., 2010, Roubeix et al., 2011). DCMU, another PSII inhibitor commercially used under the name diuron, was chosen for its extensive use as an electron-transport inhibitor in photosynthesis studies. It's inclusion allowed us to situate our work in a larger body of knowledge.

While it is well known that atrazine and other PSII electron transport inhibitors are competitive inhibitors at the QB-binding site on the Dl protein (Fuerst and Norman, 1991), few studies have compared the physiological response between groups and species (Deblois et al., 2013) nor have these effects been studied in natural communities at environmentally relevant concentrations. Through these experiments we sought to: 1) identify concentrations at which these molecules have effects on the photophysiology and growth of freshwater phytoplankton; 2) examine different methods that best allow to robustly identify this threshold; and, finally, 3) determine if different groups/species differ in terms of their response, which could affect phytoplankton community structures and, ultimately, the ecosystems that depend on them.

4.3 Material and Methods

4.3.1 Chemicals

Herbicides were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Stock solutions were made at the beginning of each experiment in deionized water. Solutions were warmed to approximately 35 °C and subjected to a sonication bath for several minutes in order to

solubilize the chemicals. Working solutions were obtained through serial dilution. Treatment levels varied from 0.2 μg L^{-1} to 200 μg L^{-1} for the selected herbicides (Table 4.1).

Table 4.1: Phytoplankton species used in experiments and herbicide concentrations tested

Phytoplankton	Origin or Strain	Concentrations tested (µg L^{-1})		
		Atrazine	Metolachlor	DCMU
Natural Communities				
Massawippi December	lake	0, 0.1, 0.2, 1, 2, 10, 20, 100, 200	0, 0.2, 2, 20, 200	Not tested
Massawippi Mars	lake	0, 0.2, 2, 4, 6, 8, 14, 20, 40, 100, 200	0, 0.2, 2, 20, 200	Not tested
Montjoie December	lake	0, 0.2, 2, 4, 6, 8, 14, 20, 40, 100, 200	0, 0.2, 2, 20, 200	Not tested
Montjoie May	lake	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 4, 8, 20	Not tested
Cryptophyceae				
Cryptomonas sp.	CPCC 336	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Cyanophyceae				
Anabaena flos-aquae	CPCC 67	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Microcystis aeruginosa nts	CPCC 124	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Microcystis aeruginosa ts	CPCC 299	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Synechococcus leopoliensis	CPCC 102	0, 0.2, 2, 4, 8, 14, 20	0, 20, 200	0, 0.2, 2, 8
Bacillariophyceae				
Asterionella formosa	CPCC 692	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Fragilaria crotenensis	CPCC 269	0, 0.2, 2, 4, 6, 8, 14, 20	0, 2, 20, 200	0, 0.2, 2, 4, 8
Chlorophyceae				
Scenedesmus obliquus	CPCC 157	0, 0.2, 2, 4, 8, 14, 20	0, 20, 200	0, 0.2, 2, 8
Chlorella kessleri	CPCC 266	0, 0.2, 2, 4, 8, 14, 20	0, 20, 200	0, 0.2, 2, 8
Neochloris oleoabundans	UTEX 1185	0, 0.2, 2, 4, 8, 14, 20	0, 20, 200	0, 0.2, 2, 8

4.3.2 Cultures

All experiments were conducted on phytoplankton grown in 6 mL glass vials maintained under controlled light and temperature conditions. Fourteen different cultures were grown (see

Table 4.1 for species and origins), of which ten were single species from four different classes and four were natural communities.

4.3.3 Individual strains

Cultures were grown under a sinusoidal light cycle with day length representative of the summer solstice around the 45th parallel North. Before transferring to the vials, cultures were diluted with culture media (Bold's basal medium [BBM] for *Microcystis, Neochloris* and *Scenedesmus*, modified CHU #10 medium for *Asterionella*, *Fragilaria*, *Cryptomomas*, modified BG-11 medium for the other cultures, Andersen 2005). After an average of a week of exponential pre-growth, cultures were diluted sufficiently to obtain strong chlorophyll *a* fluorescence signals at the most sensitive FRRF setting. A volume of 5 mL was then transferred to 6 mL vials and to 1 mL of deionized water containing herbicide solution to obtain the following final concentration range: Atrazine [0 to 20 µg L], metolachlor [0 to 200 µg L], DCMU [0 to 8 µg L] (Table 4.1). These concentrations were chosed based on a short series of initial experiments. Each treatment was repeated in triplicates. The experimental set-up allowed culturing 72 vials under controlled light and temperature conditions. In the first experiments, three cultures were tested simultaneously; first with concentrations of atrazine then with a second experiment of metolachlor and DCMU (*Anabaena*, *Microcystis* non-toxic strain [nts], *Microcystis* toxic strain [ts], *Cryptomonas*, *Fragilaria*, *Asterionella*). For the last experiments, 2 cultures were tested at a time with all three molecules tested simultaneously (but fewer treatment levels). Experiments lasted 7 to 10 days. Temperatures were maintained at 18˚C and the maximum light intensity at midday was 100 µmol/m^2/s. Vials were covered with open caps to allow gas exchange

4.3.4 Natural Communities

Weeklong experiments with natural communities from 2 lakes in the Eastern Townships (Québec, Canada) (Lac Massawippi in December and March and Lac Montjoie in December and May, Québec, Canada) were conducted in a similar fashion to the culture experiments using metolachor and atrazine. Lake surface water was collected in an autoclaved bottle, filtered with a 100 µm Nitex screen to remove most zooplankton, and acclimated in the laboratory to experimental conditions for two days in 10% BBM media before being transferred to 6 mL vials. While this likely has important effects on the structure of the

communities tested, selecting the species best adapted to the laboratory, it was necessary as lake water directly transferred to the sample vials had consistently low the variable fluorescence to maximum fluorescence ratio (F_v/F_m see below). These values that increased after minimum of two days of lab acclimation under continuous slow agitation. The light and temperature conditions were chosen to be most representative of those in the environment based on in-situ measurements of light and temperature (Table 4.2).

4.3.5 Measurements

a) Chlorophyll *a*

On the last day of the experiment, chlorophyll *a* triplicates for each sample (~200 µL) were extracted in 2 mL 3/2 (v/v) acetone 90%-DMSO (MacIntyre and Cullen 2005) and measured using a Trilogy fluorometer (Turner Designs, USA) fitted with a Chlorophyll Non-Acidification module (Turner Designs, USA). Twenty minutes were sufficient to extract pigments before measurement, with the exception of *Scenedesmus* and *Neochloris*, which required respectively 45 and 120 minutes to extract (comparison of sample volumes did not suggest solvent evaporation influenced results, data not shown). Samples were lightly vortexed before fluorescence measurements.

b) Cell counts

Another 200 µL of the culture was mixed in 9.8 mL isoton (0.9 w/v NaCl in H_2O) and analyzed on a Multisizer 4 Coulter Counter (Beckman Coulter, USA) using a 70 µm aperture. For *Microcystis* and *Anabaena*, samples were processed using the FlowCAM (Fluid Imaging Technologies, USA) with measurements only taken on a subset of treatment levels. For natural phytoplankton communities, aggregate samples for each treatment level were analysed using the FlowCAM.

c) FRRF measurements

FRRF measurements were taken daily, 30–60 minutes after the initiation of the daily light cycle using a LIFT-FRR fluorometer (Soliense, USA). Vials were kept in the dark for 20 minutes before being gently mixed by inversion, inserted into the fluorometer and measured under a protocol of 100 flashlets over 100 µs followed by a relaxation sequence of 300 µs. For cyanobacteria the induction protocol included 120 flashlets over 120 µs, as saturation of the

fluorescence signal was not obtained with 100 flashlets. Variables of interest included the minimum and maximum fluorescence (F_o and F_m), often used as a proxy for algal biomass, the variable fluorescence to maximum fluorescence [$(F_m-F_o)/F_m = F_v/F_m$] ratio, representing the maximum quantum yield of change separation, a reduction of which is often interpreted as a measurement of stress, the apparent size of the PSII reaction centers antennae (σ) and the probability of energy transfer between reaction centers (P) which influences the rate at which the reaction centers are filled. We also studied the rates of re-oxidation of PSII reaction centers (τ_1, τ_2, τ_3). Positions in the set-up were randomly assigned to account for any variation of light and temperature within, though the sequence of measuring the fluorescence was serial. Daily measurements were performed in less than an hour.

4.3.6 Statistical analyses

For the FRRF data, the values of the parameters of interest were analyzed for two individual days, the first and the last of each experiment. We also considered the average parameter value over the course of the experiment. For extracted chlorophyll *a* concentrations and cytometry analysis on the FlowCAM and Coulter counter, we only considered the last day of the experiment.

a) Testing for lowest observed effect concentrations (LOEC) of individual parameters

Concentration-response curves

Concentration-response models (CRM) for each time point or average response considered were fitted with the drc package (Ritz et al., 2015) in R (R Core Team, 2018). A number of models were considered and the best model was selected by Bayesian inference criterium (BIC). When multiple models fit comparatively well, we strove to reduce the number of different models considered altogether, and prioritized, in order: The shifted Michaelis Menten model (3 parameters), the shifted asymptotic regression model (3 parameters), the type 1 Weibull (4 parameters), and the Brain-Cousens hormesis model (5 parameters). From the selected model, we extracted predicted values and confidence intervals to determine changes from the baseline while accounting for propagation of errors. We also identified the concentration at which the predictive interval no longer overlapped with that of the null

treatment, which we considered as the maximum acceptable toxicant concentration value (MATC).

Comparing multiple means

As ranges of herbicides tested were insufficient to induce a maximal effect for many experiments, limiting the fitting of DRM, we also considered analysis of variance of the different treatment levels. A MANOVA was conducted for each day using all parameters. If significant differences were found, an ANOVA analysis was conducted for each parameter. When significant differences were found, post-hoc analysis were conducted. Dunnett's test was conducted to determine which levels of treatment differed significantly from the controls. Conditions of normality (Shapiro test) and homogeneity of variance (Levene's test) were generally met, but as with most t-statistic tests, Dunnett's test should be generally robust to small deviations from ideal test conditions and we decided to keep the few tests that did not fulfill the assumptions.

Particle analysis and extracted chlorophyll *a* concentrations showed greater heteroscedasticity and thus we used the procedure developed by Herberich et al. (2010) to determine differences between groups. This method makes no assumptions regarding distributions, sample sizes or variance homogeneity. We considered the following variables: Extracted chlorophyll *a* concentration, particle counts per volume, particle area per volume and particle volume per area (estimated as spherical volume for Coulter counts using the lower bin limit for diameter and calculated using the filled area and the ESD volume for measurements provided by the FlowCAM software [ESD measurements are calculated based on the mean of 36 feret measurements taken around the particle as filled area using the FlowCAM]. We also considered the mean sample diameter, using the lower bin limit for Coulter counted samples and mean area calculated by ESD for FlowCAM analysed samples). To determine the goodness of fit of our models, we relied on generalized least square (GLS) modelling from the mgcv package (Wood 2017) in R, incorporating weights per level of treatment, which corrected for patterns in the residuals. In GLS, rather than minimize the simple sum of squares error, observations are weighed in a way that best accounts for shared bias and inherent noise. This model was compared through analysis of variance to the null model using maximum

likelihood (as REML is unsuitable for comparing nested models with varying fixed components; Faraway, 2006).

The minimum concentration showing a significant change from the baseline was extracted for each culture and parameter (PSII, extracted chlorophyll *a* or particulate). We then calculated the MATC, as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) (TenBrook et al., 2009).

b) Multivariate analysis—Principal Response Curve

In addition to considering the effects of treatments on various FRRF parameters at specific time-points we also considered the effects of treatment on all parameters simultaneously, for the duration of the experiment. Principal response curve (PRC) analysis was developed to analyse temporal changes in communities to multiple levels of a toxicological treatment simultaneously (Van den Brink & Ter Braak 1999). We adapted the method to study the changes in the fluorescence parameters. This allows avoiding the pitfalls of pseudo-replication while analysing the full dataset, accounting for time-dependent changes. The PRC was run using the PRC() function in the vegan package (Oksanen et al., 2019) in R using the scaled parameters of interest. Herbicide concentrations were used as discrete treatment levels with the day since the beginning of the experiment as time points. We then ran an ANOVA permutation test with vial position as a stratum considering that these were part of a series. To test for the significance of the PRC across days, we ran a redundancy analysis of the scaled fluorescence parameters against the levels of concentration, testing the significance of the first RDA axis by permutation. Finally, we ran an ANOVA on the principal component analysis (PCA) result of the scaled parameters using Dunnett's test to test for the lowest significant concentration.

4.4 Results

4.4.1 Effects of PSII inhibitors on PSII fluorescence

Initial fluorescence parameters varied between experiments (Table 4.2) but were generally representative of healthy phytoplankton for all experiments. PSII inhibitors had clear effects on FRRF parameters. Significant effects were observed from the first day of measurement and were in most cases maintained throughout the experiment. When considering the effects at a

fixed concentration of 10 µg L^{-1} based on concentration-response models, DCMU was shown to be a much more potent PSII inhibitor than atrazine, showing larger changes in parameter values (Fig 4.1). While maximum and minimum fluorescence increased in most cases, F_v/F_m generally decreased in chlorophyceae, bacillariophyceae and natural lake communities, as the increase in initial fluorescence (F_o) was more important than that of the maximal fluorescence (F_m). For cyanophyceae and cryptophyceae, slight increases in F_v/F_m were observed for atrazine treatments. While, initially, similar increases were also observed for DCMU treatments in these groups, by the end of the experiment, treatments showed decreasing values for these parameters. Increases in σ and decreases in the probability of electron transfer (P) were consistently found for atrazine and DCMU treatments, as were increases in the rates re-oxidation of reaction centers, although responses were more variable for cyanophyceae where decreases were at times observed. When compared to the effects on extracted chlorophyll *a* concentrations, changes in the probability of electron transfer and re-oxidation rates as measured by in-vivo fluorescence were far more sensitive in showing the effects of PSII inhibitors. Interestingly, cyanophyceae presented small increases in extracted chlorophyll *a* concentrations. In contrast, metolachlor had few effects, even at the highest concentrations tested (200 µg L^{-1}, data not shown).

Table 4.2: Average baseline FRRF parameter values at the start of the experiment

Culture	Molecule [A=Atrazine;M=Metolachlor]	Temp (°C)	Irradiance [µmol m^{-2} s^{-1}]	F_o	F_m	F_v/F_m	σ (Å2 q-1)	*P*	τ1 [µs]	τ_2 [µs]	τ_3 [ms]
Massawippi December	A, M	12	60	111	222	0.5	516	0.44	1044	6827	1.60 x 10^5
Massawippi March	A, M	5	20	98	161	0.39	356	0.06	1913	1.00 x 10^4	2.46 x 10^5
Montjoie December	A, M	7	60	102	175	0.42	436	0.4	820	4160	1.24 x 10^5
Montjoie May	A, M	13	100	113	200	0.43	612	0.52	928	8762	1.77 x 10^5
Cryptomonas sp.	A / DCMU, M	18	100	47/48	100/101	0.53/0.53	340/344	0.45/0.45	1548/ 1879	1.00 x 10^4/1.18 x 10^4	1.74 x 10^5/ 2.48 x 10^5
Anabaena flos-aquae	A / DCMU, M	18	100	28/50	38/81	0.27/0.38	80/74	0.23/0.32	527/ 551	5873/ 4662	2.36 x 10^5/ 1.86 x 10^5
Microcystis aeruginosa nts	A / DCMU, M	18	100	27/38	50/66	0.46/0.43	75/68	0.38/0.33	687/ 654	6705/0 4766	4.86 x 10^5/ 4.21 x 10^5
Microcystis aeruginosa ts	A / DCMU, M	18	100	29/31	49/39	0.42/0.21	71/63	0.35/0.16	671/ 861	6491/ 7186	7.47 x 10^5/ 7.21 x 10^5
Synechococcus leopoliensis	A, DCMU, M	18	100	37	50	0.25	100	0.03	1414	1.32 x 10^4	3.73 x 10^5
Asterionella formosa	A / DCMU, M	18	100	111/61	220/123	0.50/0.51	720/703	0.48/0.52	882/ 771	4551/ 4635	7.90 x 10^4/ 9.54 x 10^4
Fragilaria crotenensis	A / DCMU, M	18	100	87/35	183/78	0.52/0.55	497/424	0.38/0.44	1057/ 889	1.16 x 10^4/9632	1.83 x 10^5/ 2.74 x 10^5
Scenedesmus obliquus	A, DCMU, M	18	100	75	183	0.59	392	0.47	640	3470	1.10 x 10^5
Chlorella kessleri	A, DCMU, M	18	100	79	207	0.62	377	0.46	487	2589	6.54 x10^4
Neochloris oleoabundans	A, DCMU, M	18	100	84	200	0.58	398	0.44	791	8393	1.30 x 10^5

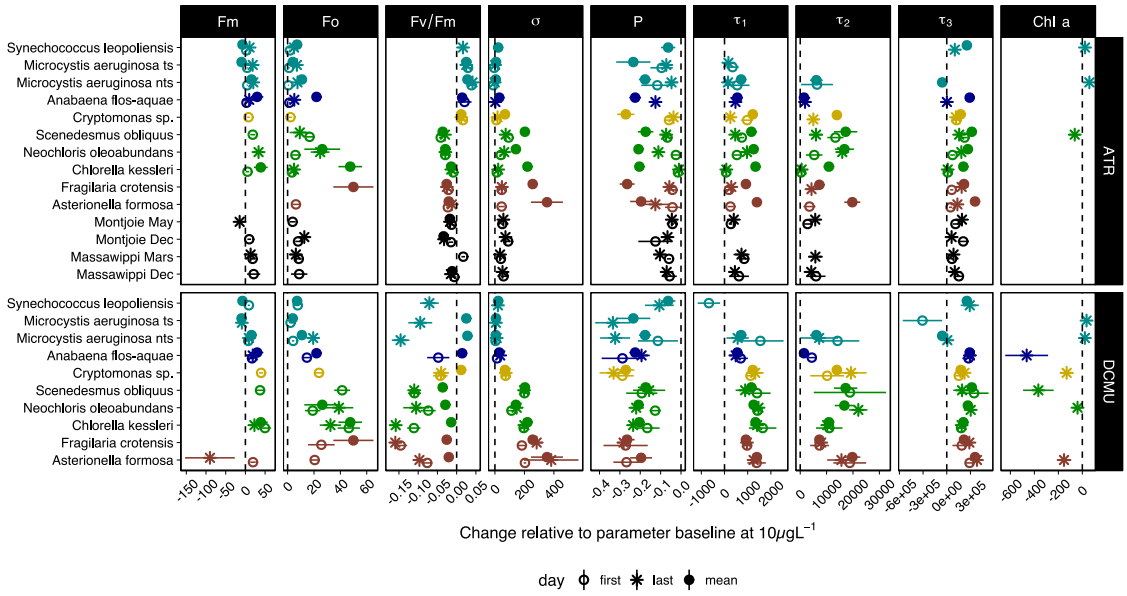

Figure 4.1: Mean effects [with standard errors] of atrazine [ATR] and DCMU on FRRF parameters and extracted chlorophyll a at a concentration of 10 µg L^{-1}. Effects on the first and last day of the experiment as well as the average across the experiment are shown. Non-significant effects are omitted.

4.4.2 Maximum acceptable toxicant concentrations

One of the main objectives of this study was to determine at what concentrations of herbicides we could observe effects on the photo-physiology of phytoplankton and how these endpoints compare to the more traditional endpoint of growth as estimated by chlorophyll *a* concentrations. When comparing the minimum significant values we found that FRRF parameters were much more sensitive endpoints than extracted chlorophyll a or cell counting/imaging [Fig 4.2]. While the latter endpoints were only significant within the chlorophyceae and cyanophyceae classes, all classes tested were found to be significantly impacted at the concentrations tested when considering the FRRF endpoints through multivariate analysis. When comparing individual FRRF parameters, Dunnett's test found significant effects at lower concentrations than concentration-response curves [Fig 4.3]. The lowest DRC maximal acceptable toxicant concentrations (MATCs) were found quasi-exclusively for fluorescence parameters specific to FRRF protocols. While bacillariophycea and chlorophyceae had a greater number of significant parameters at the lowest MATC concentration, all cultures tested were similarly sensitive for at least one parameter.

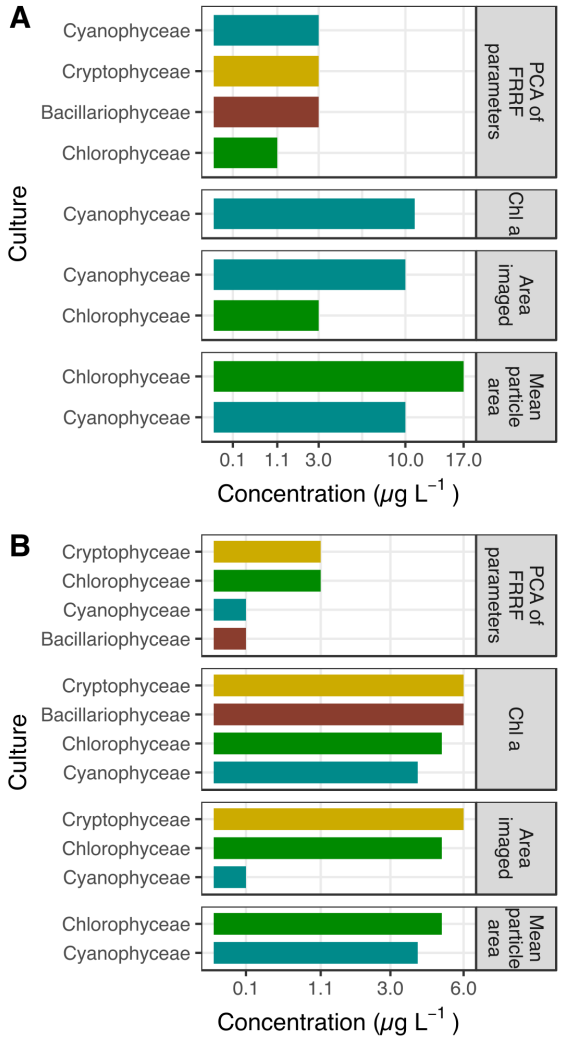

Figure 4.2: Lowest MATC found for each class of phytoplankton tested for PCA of FRRF parameters, extracted chlorophyll, area of particles imaged and average cell size on the last day of the experiment for A) Atrazine and B) DCMU. MATC values exceeding the maximum concentration tested are not shown (note: x-axis scale is square root transformed).

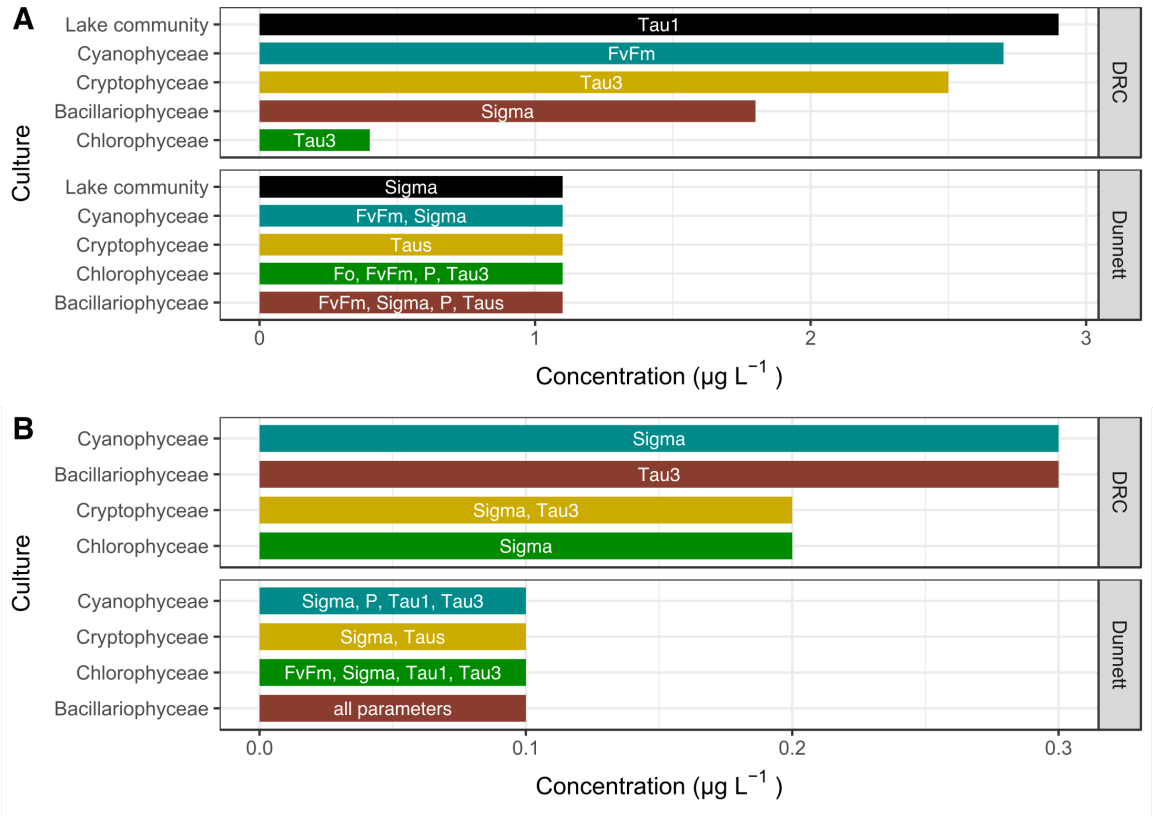

Figure 4.3: Lowest MATC values found for each phytoplankton class through the analysis of individual FRRF parameters using concentration-response curve analysis and Dunnett's test for A) atrazine and B) DCMU

We found that for all phytoplankton groups considered, minimum MATC values for PSII endpoints were far below existing guidelines and standards (Table 4.3).

The smallest MATCs for atrazine were found for individual FRRF parameters where MATCs of 1.1 were found within all classes, while DRCs of individual parameters and the ANOVA of the first PCA axis yielded on average similar ranges of MATCs (1–3 µg L^{-1}) (Fig 4.2, Fig 4.3). For DCMU, average minimum MATC concentrations were found to be 0.1 µg L^{-1} for Dunnett and 0.25 µg L^{-1} for DRC analysis, compared to a value of 4.6 µg L^{-1} found for chlorophyll *a* and cell imaging. We remark that as all experiments showed significant effects at the lowest concentration of DCMU tested, it is likely that actual MATC values are lower.

For metolachor, while significant effects were found in 80% of cases considered based on Dunnett's test, DRC only demonstrated effects on the last day, and only for bacillariophycea and chlorophyceae (Annexe A; S4.1). The average minimum concentration at which effects

were found were also higher (DRC: 123.4 µg L^{-1}; Dunnett: 34 µg L^{-1}). Comparatively, effects on chlorophyll *a* concentrations and cell imaging endpoints yielded an average MATC value of 110 µg L^{-1}.

Overall, we found strong evidence for statistically significant physiological effects of PSII inhibitors on freshwater phytoplankton physiology at concentrations not only below existing MATC values, but often below derived water quality guideline values. While there is some evidence that chlorophyceae (particularly *Scenedesmus obliquus*) might be more sensitive to atrazine, PSII inhibitors generally had comparable effects across groups.

Table 4.3: Current environmental guidelines and standards for the herbicides studied and recommendations based this current study (Concentrations in µg L^{-1})

	Atrazine	DCMU	Metolachlor
Existing freshwater guidelines and standards			
Canadian Environmental Quality Guidelines—Protection of aquatic life (chronic exposition) (CCME 1999a, CCME 1999b)	1.8	1.6*	7.8
United States Environmental Protection Agency National Recommended Aquatic Life Criteria (USEPA 2019)	NA	NA	NA
Australia and New Zealand Guidelines for fresh and marine water quality (ANZECC & ARMCANZ 2000)	13	0.2	ID
European Union Environmental quality standard: Maximum allowable concentration for Inland surface waters (EU Parliament 2013)	2	1.8	NA
Guideline values derived from current study			
Lowest observed effect concentration (LOEC)	2**	0.2	20**
No observed effect concentration (NOEC)	0.2	0	2
Maximum allowable toxicant concentration (MATC)	1.1	0.1	11
Guideline value (Safety factor of 10)	0.11	0.01	1.1

* As the CCME doesn't have a guideline value for DCMU, we report that of the province of Ontario (also used in Québec, Guay et al., 2013) (OMOEE 1994)

**For atrazine, though effects at smaller concentrations were observed on the first day (0.2 µg L^{-1}; Annexe A S4.1) we chose to retain the more commonly and consistently observed effect at 2 µg L^{-1}; idem with metolachlor and effects at 0.2 - 2 µg L^{-1}

4.4.3 Robustness of statistical methods

Concentration-response curves (not shown) produced more robust results than Dunnett's test, as Dunnett's test is more susceptible to the influence of random variation between groups. Our concentration-response curve MATC values were, however, greater than those found using Dunnett's test. This could be due to several factors, including that the values were extracted from predictive intervals rather than model confidence interval, which would require additional data points for best performance, and that our models often did not include maximal effects (data not shown).

Analysing FRRF parameter individually is time consuming and introduces the risk of increased type II error due to the sheer number of statistical tests considered. Our results suggest that multivariate analysis of FRRF parameters provides robust yet sensitive results (Fig 4.2). When considering the minimum significant concentration found for each culture group, MATCs determined by PCA analysis were found to be approximately twice as large than when considering the most sensitive individual parameters but remained smaller than MATCs derived from the literature. Likely due to the fact that metolachlor does not affect photosystem II, PCA analysis revealed fewer significant endpoints than when considering minimum and maximum fluorescence yield individually (Annexe A; S4.1). When MATC values were found, these were generally below literature MATC values but above guideline values.

4.4.4 Principal response curves

PRC analysis demonstrated that, with the exception of cyanophyceae, atrazine had statistically significant effects on PSII parameters derived from FRRF within 24 hours; these were maintained for the duration of the experiment (Table 4.4). The inertia of the PSII parameters that could be explained by our experiments was generally greatest for chlorophyceae and smallest for cyanophyceae and spring/winter natural communities, with intermediate results for bacillariophyceae, cryptophyceae and summer natural communities. When considering the proportion of the inertia that could be linked to treatments, excluding temporal changes, with over half of the inertia being attributed to PSII inhibitor treatments for spring natural communities, *Scenedesmus* and *Neochloris*, two chlorophyceae and *Microcystis*, a cyanophyceae. Comparison between atrazine and DCMU reveals that a larger proportion of

the inertia in the parameters could be explained by treatment in the case of DCMU, consistently with the lower effect concentrations for this molecule. However, when looking within a molecule tested, there is no clear association between the ability to explain changes in the parameters and the minimum concentrations with significant effects. These results suggest that while the protocol used was best suited for precisely measuring in-vivo chlorophyll *a* fluorescence of chlorophyceae and bacillariophyceae, it was sensitive enough to find significant differences in other phytoplankton groups at comparative concentrations. This suggests that it is likely adequate to use on natural phytoplankton communities.

Table 4.4: Principal Response Curve analysis: Variance explained by treatment through Redundancy Analysis (Non-significant values shown in grey)

	Inertia explained			Dunnett minimum significant difference		
Culture	mean response	first day	last day	Mean response	first day	last day
Metolachlor						
Scenedesmus obliquus	0.62	0.07	0.68	200		200
Asterionella formosa	0.38	0.03	0.24	200		2
Microcystis aeruginosa nts	0.16	0.18	0.14			
Synechococcus leopoliensis	0.15	0.21	0.02			
Chlorella kessleri	0.09	0.17	0.18			
Neochloris oleoabundans	0.09	0.16	0.15			
Cryptomonas sp.	0.07	0.08	0.11			
Microcystis aeruginosa ts	0.07	0.09	0.05			
Fragilaria crotenensis	0.07	0.07	0.11			
Massawippi Mars	0.06	0.04	0.01			
Montjoie Mai	0.06	0.06	0.03			
Montjoie December	0.05	0.02	0.05	20		
Anabaena flos-aquae	0.05	0.16	0.03			
Massawippi December	0.03	0.01	0.05			

Atrazine						
Neochloris oleoabundans	0.83	0.74	0.70	4	4	8
Chlorella kessleri	0.76	0.47	0.68	4	4	2
Montjoie December	0.71	0.52	0.53	2	6	4
Montjoie Mai	0.68	0.44	0.68	4	4	6
Scenedesmus obliquus	0.66	0.74	0.57	2	0.2	2
Massawippi Mars	0.64	0.63	0.42	4	4	4
Fragilaria crotenensis	0.63	0.48	0.52	2	8	4
Massawippi December	0.62	0.47	0.58	10	10	10
Cryptomonas sp.	0.59	0.37	0.58	4	8	4
Asterionella formosa	0.55	0.47	0.33	8	6	20
Microcystis aeruginosa nts	0.52	0.23	0.47	4		4
Microcystis aeruginosa ts	0.51	0.19	0.43	4		4
Anabaena flos-aquae	0.37	0.15	0.30	4		20
Synechococcus leopoliensis	0.30	0.18	0.09	2		14
DCMU						
Chlorella kessleri	0.91	0.91	0.87	2	2	2
Neochloris oleoabundans	0.79	0.81	0.68	2	2	8
Fragilaria crotenensis	0.74	0.68	0.61	0.2	2	0.2
Anabaena flos-aquae	0.72	0.58	0.61	0.2	0.2	0.2
Scenedesmus obliquus	0.72	0.81	0.77	0.2	2	2
Microcystis aeruginosa nts	0.68	0.36	0.50	2	2	2
Asterionella formosa	0.68	0.83	0.53	0.2	2	4
Cryptomonas sp.	0.59	0.74	0.65	0.2	2	2
Synechococcus leopoliensis	0.48	0.33	0.53	8	2	8
Microcystis aeruginosa ts	0.33	0.21	0.39	8	4	4

4.5 Discussion

Our results show that in-vivo fluorescence is a valuable tool to study the effects of PSII inhibitors on phytoplankton cultures and natural communities. We consistently found measurable effects at concentrations ten times smaller than the most conservative values in the literature. We propose the following model, which will have to be fully tested, to explain our key results pertaining to fluorescence induction on eukaryotes based on PSII dimer model (Fig 4.4). These key results are an increase of F_o, a decrease in P and an increase in the absorption cross section in the presence of the PSII inhibitor. Under standard conditions, as reaction centers are closed under successive light flashes, chlorophyll *a* fluorescence increases, when energy can no longer be shared with neighbouring reaction centers (a). When PSII inhibitors irreversibly block a small fraction of the reaction centers, the probability of energy being redirected to another reaction center and the probability of energy directly reemitted as fluorescence increases, leading to increase in F_o and decreases in P (b). The decrease in the probability of energy transfer that is observed with the FRRF protocols with increasing concentrations of PSII inhibitors reflects the loss of the sigmoidal shape during fluorescence induction. This sigmoïdicity was suggested to be due to the connectivity of reaction centers, allowing the transfer of excitation from a closed reaction center to another reaction center (Joliot and Joliot, 1964). The model for PSII excitonic connectivity remains somewhat contentious (see Stirbet 2013 for review). Alternative explanations for this raise have been proposed, notably that it is the result of the superimposition of two rises (Vredenberg 2008). Within our schematic, PSII connectivity decreases as a result of an increased baseline of closed reaction centers as the realized structure observed by the FRRF emission is that of unconnected reaction centers with larger antennae (c). The slight increase in F_m also observed in the presence of the inhibitor could arise from the prevention of reoxidation of a small fraction of the PSII reaction centers that normally occurs during the induction process (Kolber et al., 1998). PSII inhibitors impact the kinetics of reaction center relaxation at low concentrations, which has consequences for photosynthesis since it takes longer for the available reaction centers to become available for further excitation by photons. The lengthening of the relaxation rates could also be indicative of electron backflow.

Our results further suggest that under constant and low PSII inhibition, phytoplankton may adapt by investing in the creation of additional reaction centers, or larger antennae, to improve the rate of reaction. This is most evident when considering DCMU experiments where initially significant increases in F_m were associated with decreases in extracted chlorophyll *a* concentrations by the end of the weeklong experiments, suggesting that evolutionary physiological adaptations were ultimately insufficient to compensate for herbicide toxicity (Fig 4.1).

While herbicide concentrations in lakes are far less documented than those in rivers and streams, lake atrazine concentrations were measured during the 2012 National Lake Assessment (EPA-NLA) campaign (see Beaulieu et al., 2019 submitted [Chapitre 5]). While only 1% of lakes were found to have atrazine concentrations exceeding 2 µg L^{-1}, 10% of lakes had concentrations exceeding 0.2 µg L^{-1}. When looking at the more highly human impacted plains and lowlands ecoregions, this proportion raises to nearly 18% of lakes. Our results suggest that concentrations of atrazine leading to physiological acclimation of algae are currently found in US lakes.

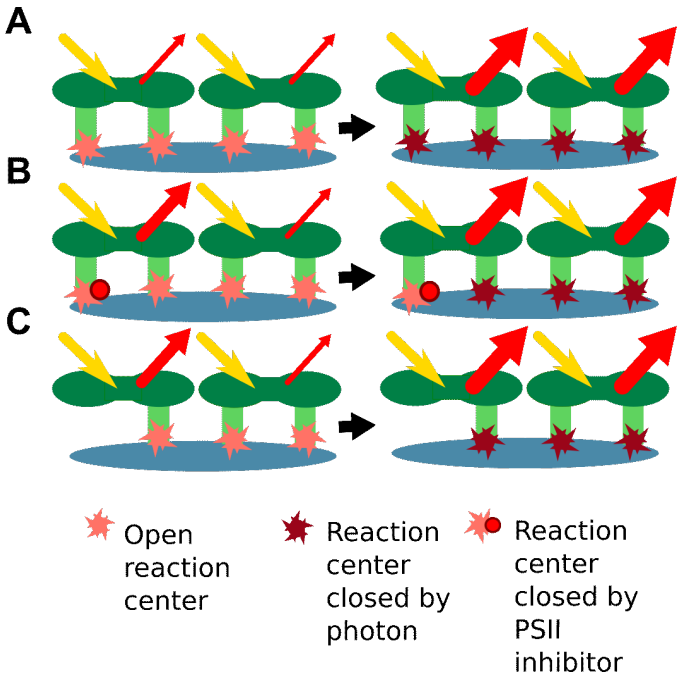

Figure 4.4: Schematic of the effects of low concentrations of PSII inhibitors on PSII (See text for details). A) Untreated PSII, B) Effect of the presence of the inhibitor, C) Apparent structure of PSII in the presence of PSII inhibitors

Our results suggest that current guidelines and standards for the protection of freshwater environments are insufficient to protect phytoplankton from the effects of PSII inhibitors in the aquatic environment (Table 4.3). Guideline values are determined by applying safety factors where increased uncertainty warrants larger factor values (100-1000), notably when using acute exposition endpoints to derive chronic exposition guidelines (Malaj et al., 2014). The Canadian province guidelines were the lowest for atrazine (1.8 $\mu g\ L^{-1}$) and metolachlor (7.8 $\mu g\ L^{-1}$), while DCMU guideline values were lowest in Australia and New Zealand. While this latter value of 0.2 $\mu g\ L^{-1}$ was an order of magnitude smaller than the other guidelines and standards, it is of the same order of magnitude as a recommended water quality guideline of 0.13 $\mu g\ L^{-1}$, obtained by applying a safety factor of 10 to a calculated MATC of 1.3 $\mu g\ L^{-1}$ (Fojut et al., 2011). Our recommended guideline values of 0.11 $\mu g\ L^{-1}$ for atrazine and 0.01 $\mu g\ L^{-1}$ for DCMU, which are based on MATC values consistently found for various phytoplankton classes and natural communities and a conservative safety factor of 10, remain over an order of magnitude smaller than the most conservative values used to guide policy. The guideline values we derived for metolachlor are, however, more consistent with existing guidelines.

It is difficult to ascertain the ecological relevance of our findings, Phytoplankton species appear capable of acclimating to the presence of herbicides. Our results suggest that, in the absence of functional effects, it might be necessary to determine how other processes within the cell are affected by the energetic expenditure required to increase the number of reaction centers. For this it might be useful to consider changes in the biochemical profiles of cultures, especially given that these will have repercussions throughout the food chain. Previous studies have shown that chemical stressors lead to changes in fatty acid content (Filimonova et al., 2016). Furthermore, the responses observed of an increase in the PSII antenna size and decrease in the transport rates suggests that the most deleterious effect of PSII inhibitors at low doses will occur at high light levels (surface or near surface) when cellular capacity to process absorbed photon will be reduced compared to uninhibited cells, such conditions are rarely tested in laboratory settings.

4.6 Conclusion

Through the use of FRRF we demonstrate that PSII-inhibiting herbicides consistently have effects on freshwater phytoplankton photo-physiology at concentrations far below concentrations affecting the most sensitive species in previous studies. Although the ecological impacts of the acclimation of phytoplankton communities to the presence of PSII inhibitors can not be assessed, our results suggest that following stronger guidelines for PSII inhibitors may be required to adequately limit the environmental effects of these herbicides.

The parameters specific to FRRF ($P, \sigma, \tau_1, \tau_2, \tau_3$) are very sensitive to PSII inhibitors and this protocol allows to detect significant effects at lower concentrations than when only considering Fo, Fm and their ratio, as is done with PAM fluorometry. Multivariate analysis of FRRF parameters performed sensibly as well as the most sensitive parameter and appears to be as robust as intrapolating significant concentrations from concentration-response curves.

As has been suggested by Kruskopf and Flynn (2005), maximal chlorophyll *a* fluorescence is not a good estimate of biomass of algae under stress conditions. We further show support that in the case of herbicides blocking the QB site of photosystem, low concentrations can induce an increase in chlorophyll *a*, which can mask negative effects at lower concentrations, as the oxidized state of the plastoquinone pool mimics low light conditions (Escoubas 1995). The increase in extracted chlorophyll *a* fluorescence was most remarkable in cyanobacteria but may be confounded by the overlap of phycobilisome fluorescence with that of chlorophyll *a* (Campbell 1998).

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CHAPITRE 5

L'ATRAZINE DANS LES LACS À L'ÉCHELLE DU PAYSAGE

Avant-propos

Auteurs et affiliation :

M. Beaulieu : étudiante au doctorat, Université de Sherbrooke, Faculté de génie, Département de génie civil.

H. Cabana : professeur, Université de Sherbrooke, Faculté de génie,
Département de génie civil

Z.E. Taranu : chercheuse scientifique, Division de la recherche sur les contaminants aquatiques, Environnement et Changement climatique Canada

Y. Huot : professeur, Université de Sherbrooke, Faculté des lettres et sciences humaines, Département de géomatique appliquée

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Contribution au document :
Cet article contribue à la thèse en étudiant l'importance de la contamination des lacs à grande échelle spatiale par un herbicide couramment utilisé. Les modèles développés permettent de mieux comprendre les facteurs influençant la contamination des lacs et prédire le risque de contamination de sites qui n'ont pas été échantillonnés.

Résumé français :
Malgré l'omniprésence de la contamination des eaux de surface par l'atrazine en Amérique du Nord, peu d'analyses spatiales ont été développées pour modéliser cette contamination à l'échelle du paysage. À l'aide des données de l'évaluation nationale des lacs de l'EPA des États-Unis de 2012, des bases de données CropScape du ministère de l'agriculture des États-Unis de 2012 et des centrales globales HydroLAB HydroLAKES, nous avons développé des modèles prédictifs binomial-gamma et de régression LASSO pour l'atrazine dans plus de 900 lacs des États-Unis contigus. Alors que les concentrations observées étaient généralement faibles, avec une valeur moyenne de 0,17 µg L^{-1}, l'atrazine était détecté dans 32% des lacs américains. Le prédicteur les plus importants de la détection d'atrazine dans les lacs étaient la présence de cultures de maïs et de soja dans le bassin versant. La masse surfacique estimée de l'application d'atrazine (kg d'atrazine km^2) dans le bassin versant était le facteur prédictif le plus important de la concentration d'atrazine retrouvée lorsque la molécule était détectée.

Toutefois, en l'absence de cette dernière donnée, les modèles prenant uniquement en compte la proportion de maïs ou de soja dans le bassin versant expliquent une proportion similaire de variance (59% vs 60% pour tous les lacs; 44% vs 46% dans l'écorégion des Plaines et des basses terres). Les variables de qualité de l'eau associées à l'eutrophisation étaient associées à des niveaux accrus de contamination par l'atrazine, tandis que des températures de l'eau plus froides et des lacs et paysages naturels étaient associés à des niveaux de contamination plus faibles. Nos modèles ont expliqué jusqu'à 75% de sa variance sur un paysage hétérogène dans l'espace et dans le temps, démontrant que des changements dans les pratiques d'utilisation des sols peuvent aider à atténuer la contamination par l'atrazine dans les lacs américains.

Note : À la suite des corrections demandées par les membres du jury, le contenu de cet article diffère de celui qui a été soumis.

5.1 Abstract

Despite the ubiquity of atrazine herbicide contamination of surface waters in North America, few spatial analyses have been developed to model its occurrence across the landscape. Using data from the 2012 U.S. EPA National Lake Assessment, the 2012 U.S. Department of Agriculture CropScape and the Global HydroLAB HydroLAKES databases, we developed predictive binomial-gamma hurdle models and LASSO regression models for atrazine for over 900 lakes from the contiguous United States. While the concentrations observed were generally low, with a mean value of 0.17 µg L^{-1}, atrazine was detected in 32% of US lakes. The most important predictors of the detection of atrazine in lakes was the presence of corn and soy culture in the watershed. The estimated areal weight of atrazine application (kg atrazine km^2) in the watershed was the most important predictor of the concentration of atrazine when the molecule was detected however, models considering only the proportion of corn or soy in the watershed explained a comparable amount of the total variance of the hurdle models (59% vs 60% for all lakes; 0.44% vs 0.46% in the Plains and lowlands ecoregion). Water quality variables associated with eutrophication were associated with increased levels of atrazine contamination while cooler water temperatures and natural lakes and landscapes were associated with decreased levels of contamination. Our models explained as much as 75% of its variance across a spatially and temporally heterogeneous landscape, showing that changes in key land use practices may help mitigate atrazine contamination in U.S. lakes.

5.2 Introduction

Atrazine is the second most used pesticide in the United States after glyphosate (Atwood and Paisley-Jones 2017) and is an essential additive in corn production as conducted through industrial agriculture practices. Relatedly, atrazine is also an important aquatic micro-pollutant; amongst the molecules commonly screened, it has long been one of the most frequently detected in North American waterbodies (Solomon et al., 1996, Baldwin et al., 2016). The relationship between the volume of atrazine applied on land and the downstream concentrations in inland lakes and reservoirs, however, is difficult to determine. Although herbicide application volumes can be estimated from the volumes purchased and the area of cultivation, once applied, the environmental fate of these additives can be quite variable

among sites due to differences in soil, water and air transport. Consequently, atrazine can be transported hundreds of miles from where it is applied depending on the transport route (Glotfelty et al., 1987; Chernyak et al., 1996). In soils, although its half-life can be as short as 5 d (Vryzas et al., 2012), the persistence of atrazine residues over several decades suggests that realized half-lives in the environment are significantly longer than predicted (Jablonowski et al., 2011, Vonberg et al., 2014a). This would explain why atrazine remains detected in German aquifers 21 years after its ban in 1991 (Vonberg et al., 2014b). Reported half-lives of atrazine in surface waters vary wide widely from 3.2 d (Kosinski 1984) to 168 d (Bacci et al., 1989) depending of the conditions). Because of its widespread use, distribution and potential persistence, atrazine contamination of freshwater ecosystems is likely to remain a concern for decades to come, and yet may be a difficult endpoint to predict.

Atrazine is a likely endocrine disruptor in humans, based on amphibian and mammalian models (Hayes et al., 2010; Breckenridge et al., 2015) and has been associated with cytotoxicity in human and animals cells (Liu et al., 2006, Huang et al., 2014), as well as oxidative stress (Jin et al., 2014) and dopaminergic effects (Li et al., 2015) in animal models. Although the risks beget by the presence of low concentration of atrazine in water bodies used as drinking water sources and for recreation will likely never be fully ascertained, monitoring and subsequent predictive modelling can identify areas of potential concern and factors most associated with the presence of atrazine. The cumulative impact of atrazine and other anthropogenic pressures such as eutrophication due to agricultural practices and climate change may represent a further risk to the health of these systems.

While atrazine contamination of streams, rivers and groundwater of the contiguous United States has been monitored through the USGS National water-quality Synthesis project (NAWQ) since 1992 (Toccalino et al., 2014, Ryberg et al., 2015), no comparative surveys have existed for lakes until the second campaign of the U.S. Environmental Protection Agency's (USEPA) National Lakes Assessment (NLA) in 2012 (USEPA 2016). This dataset presents an unprecedented opportunity to explore the extent of our ability to predict where atrazine could be found as well as its level of contamination, and how this may vary in response to land use and climate (i.e. hydrology) change, and local lake parameters (e.g. physico-chemical parameters). As environmental micro-pollutant sample extraction and

processing can be a difficult and costly endeavour, notably with regards to complex matrixes (Souza-Silva et al., 2015), predictive lake models would also allow us to extend our knowledge to sites that have not been sampled. While watershed hydrological models can be used to estimate the fate of atrazine within watersheds (Vazquez-Amabile et al., 2006; Larose et al., 2007), we are aware of two models that have been developed to predict the spatial distribution of atrazine in aquatic environments (Stackelberg et al., 2012; Yun and Qian 2015). Stackelberg et al., (2012) modelled atrazine concentrations in shallow groundwater, finding that atrazine concentrations are less controlled by dispersion and transformation of the molecule than by the history of atrazine use in relation to the groundwater age. Yun and Qian (2015) found crop cover to explain the spatial variation in temporal atrazine trends in streams and lakes, with increasing concentrations through time in the south with an expansion of crop land and decreasing or stable concentrations in northern regions of the U.S. where crop cover was initially high.

Due to the uniquely anthropogenic origin of atrazine we considered the most likely variables to have an influence on atrazine concentrations in lakes would be related to its use in the watershed of a given lake. Atrazine is used quasi-exclusively on corn crops (USGS, https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2016&map=ATRAZINE&hilo=L) and lakes within watershed with a high density of corn culture should have higher atrazine concentrations. It should be noted that it is common agricultural practice to yearly alternate corn and soy such that the presence of soy on any given year can be indicative of the presence of corn on a previous year. Lakes may act as retention basins for atrazine, with subsequent immobilisation of the herbicide in the sediments (Qu et al., 2017). Because of this, water retention time might be important in predicting atrazine concentrations, with long retention times being associated with lower water-column concentrations, as atrazine is adsorbed to the sediments in addition to being degraded in the water column through microbial transformation (e.g. oxidative dealkylation and hydrolysis; Fenner et al., 2013). We also considered physical lake parameters and water chemistry parameters that might have an influence on the fate of atrazine in the water column. While the co-occurrence of nutrient and herbicide pollution from agricultural sources are likely to make the former a predictor of the occurrence of the second, low pHs could be associated with greater recalcitrance of atrazine, comparably to what is observed in soils (Houot et al 2000; Krutz et al., 2010). Higher

salinities have also been associated with decreased atrazine degradation (Solomon et al., 1996; Lin et al 2008). Physical parameters, notably temperature may also be predictors. In soils, warmer temperature increases atrazine biodegradation up to 30°C (Strong et al., 2000; Krutz et al., 2008) but also favours atrazine sorption to soil surfaces (McGlamery and Slife, 1966; Yue et al 2017). Lake properties such as area, depth and water column stability could influence the amount of atrazine arriving in the lake and its distribution in the water column. The U.S. contains a large number of human-altered lake systems and it has been shown that these different types of systems can impact biotic and abiotic conditions (Beaulieu et al., 2013; Hayes et al., 2017) and we considered lake type as a possible predictor. We also considered the day of the year, as seasonal effects due to punctual timing of atrazine applications could influence the concentrations found in the aquatic environment. By analysing the influence of this large number of variables we sought to explore their respective importance in predicting atrazine concentrations in lakes.

A challenge to modelling environmental contaminant concentration data is that such data tends to be right-skewed and comprising a large number of samples that are below the detection values. Substitutions, imputation methods and non-parametric models have been used to develop regression models for distributions with below-detection values but such methods introduce biases, notably when non-detects make up the bulk of the data (Helsel 2015). One approach to avoid the pitfalls of such methods is to analyze the data sequentially using a hurdle (or two-part) model, firstly seeking to explain which lakes are exceeding (yes or no) the detection limit, and secondly estimating concentrations above the detection limit. Although interest for such models for continuous data is not new (Aitchison, 1955) they have not been heavily adopted for continuous data, in contrast to the literature analysing count data (Mullahy 1986). Recent work by Taranu et al., (2017), in which microcystin models were developed, has demonstrated the value of the method for continuous environmental data. We sought in this study to develop empirical hurdle models explaining broad-scale patterns of atrazine in lakes at the continental scale, considering landscape and within-lake processes.

5.3 Methods

5.3.1 The dataset

We analyzed data from 1135 lakes and reservoirs in 48 U.S. states sampled in 2012 through the NLA survey. The NLA's objectives are to identify the current health status of lakes and reservoirs in the U.S. and assess how this has changed through time, and, to this end, lakes sampled are randomly selected to be representative of U.S. lakes (Pollard et al., 2018). During this second survey (the first occurring in 2007) surface water atrazine concentrations were measured. In each site, integrated water samples were collected water from the euphotic zone (calculated as twice the Secchi depth), up to depths of 2 meters. Atrazine containing samples were conserved in 60 mL white HDPE bottles and stored on ice for up to one week before shipping for analysis. Quantification was performed using the Abraxis Atrazine ELISA kit (Abraxis LLC).

5.3.2 Predictor variables

We considered a number of variables in explaining atrazine concentrations in lakes (Table 5.1; Fig 5.1). These variables were transformed to meet conditions of normality and heteroscedascity when possible. The variables considered were varied in type and data origin and were grouped in the following broad categories:

a) Land-use and atrazine use intensity

We used the 2012 US Department of Agriculture (USDA) CropScape data layer (https://nassgeodata.gmu.edu/CropScape/) to calculate the land use within a lake's watershed using the NLA watershed polygons as the limits. We calculated a number of watershed specific land use parameters (expressed as the fraction of the total land cover in the watershed, table 5.1) including total agriculture (P_{Agr}), developed land (P_{Dev}), natural landscape (P_{Nat}) and water/perennial ice (P_{Wat}). As atrazine is used quasi-exclusively on corn crops, we also considered the area of corn culture (P_C). However, given that the dominant cropping system in the US Corn Belt, where over 85% of US corn is cultivated, on a 2-year corn-soybean rotations (Grassini et al., 2015), we likewise considered the sum of soybeans and corn cultures (P_{CS}).

The 2012 United States Geological Survey (USGS) National Water Quality Assessment (NAWQA) county-level scale atrazine use estimate data (https://water.usgs.gov/nawqa/pnsp/usage/maps/index.php) was used to determine the estimated area density of atrazine application in direct vicinity of the lake (EA_L), the estimated average area density of atrazine application in the watershed (EA_W) and the mass of atrazine applied in the watershed (EA_M). This data is obtained by calculating the median pesticide-by-crop use rate at the county level using farm surveys of pesticide use and estimates of harvested crop acres. These rates are then applied to the harvested acreage of each crop within the county (for detailed methods see Thelin and Stone 2013, Baker and Stone 2015).

b) HydroLAKES data

The hydroLAKES 1.0 database (http://www.hydrosheds.org/page/hydrolakes) aims to provide shoreline polygons for lakes around the world with a surface area of at least 10 ha using remote sensing (methods provided in Lehner and Messager 2016). The database additionally provides shoreline length, average depth, water volume and residence time attributes. Lake depths were estimated with elevation data while hydraulic residence time was estimated from the global integrated water model WaterGAP (Messager et al., 2016). We merged the NLA and hydroLAKES dataset for all polygons showing overlap. We further eliminated lakes for which we found differences greater than a factor of 50 for common variables (watershed area, lake area, and elevation).

c) Water chemistry (NLA 2012)

We chose a number of parameters with potential ecological effects on the atrazine concentrations in lakes (Table 5.1), including nutrient concentrations (total nitrogen [TN], total phosphorus [TP], ammonium [NH_4], and total nitrogen to total phosphorus ratio [TN:TP]), and additional water quality parameters (turbidity [Turb], conductivity [Cond], colour [Color], dissolved organic carbon [DOC], acid neutralizing capacity [ANC] and pH). We further considered the concentration of select ions (Cl^-, Na^+, Ca^{2+}, K^-, SO_4^-) and silica (SiO_2).

d) Physical parameters (NLA 2012)

Selected spatial and landscape parameters included elevation [Elev], latitude [C_{Lat}], and longitude [C_{Lon}], as well as the Wadeable Streams Assessment ECO9 ecoregions. These were

chosen to characterize the broad spatial distribution of lakes in the dataset. Lake area [A_L], watershed area [A_W], lake to watershed ratio [$A_{L:W}$], lake depth (D), surface water temperature (T_S) and the Brunt-Väisälä water-column stability index (N) were also considered. We also considered the type of water body. The 2012 NLA campaign included a number of categories for this variable: natural lakes, natural lakes supported by flow diversions (enhanced), man-made impoundments, man-made impoundments whose initial purpose has been abandoned for many decades, and reservoirs used for hydro-electricity, flood control and active irrigation. As there were only 4 abandoned impoundments and 44 enhanced natural lakes, we chose to merge these groups into that of natural lakes (i.e., grouping natural lakes, enhanced natural lakes and abandoned impoundments), which resulted in three lake type categories: lakes, impoundments and reservoirs. Day of the year [D] was considered to control for seasonality.

Table 5.1: Distribution and transformation of atrazine concentrations and considered predictor variables

Category (Dataset)	Variable	Units	min value (min non-zero value)	median value	mean value	max value	Data transform.
Atrazine (NLA 2012)	Atrazine [Below/Above detection limit] (ATR_PA)	binary		Below: 648; Above: 302			
	Atrazine concentration [detection limit (dl) = 0.046 µg L^{-1}] (logATR)	µg L^{-1}	0.046	0.046	0.167	9.7	$\log_{10}(x)$-$\log_{10}(dl)$
Ecoregion (NLA 2012)	Eastern Highlands	NAP=Northern Appalachians; SAP=Southern Appalachians					
	Plains and Lowlands (PlnLow)	CPL=Coastal Plains; NPL=Northern Plains; SPL=Southern Plains; TPL=Temperate Plains; UMW=Upper Midwest					
	Western Mountains and Xeric	WMT=Western Mountains; XER=Xeric West					
Water quality (NLA 2012)	Total phosphorus (TP)	µg L^{-1}	4	44	123	3636	$\log_{10}$ (X)
	Total nitrogen (TN)	mg L^{-1}	0.01	0.63	1.20	54	$\log_{10}$ (X)
	pH		2.83	8.32	8.13	10.37	
	Silicate (Si)	mg L^{-1}	0.02	6.18	10.72	255	$\log_{10}$ (X)
	Sodium (Na)	mg L^{-1}	0.09	7.68	129.56	2.99×10^{-4}	$\log_{10}$ (X)
	Potassium (K)	mg L^{-1}	0.04	2.24	14.07	3376	$\log_{10}$ (X)

	Sulfate (SO_4^-)	mg L^{-1}	0.03	7.68	232.51	47325	$\log_{10}$ (X)
	Turbidity (Turb)	NTU	0.01	2.47	9.07	404	$\log_{10}$ (X)
	Chloride (Cl)	mg L^{-1}	0.04	1.84	55.66	18013	$\log_{10}$ (X)
	Calcium (Ca)	mg L^{-1}	0.12	20.7	30.06	595	$\log_{10}$ (X)
	Aluminium (Al)	mg L^{-1}	0 (0.001)	0.004	0.02	2.48	$\log_{10}$ (X)
	Acid neutralizing capacity (ANC)	µeq L^{-1}	-3361	1554	2786	3334	$\log_{10}$(X+ min(X)+1)
	Ammonium (NH_4)	mgN L^{-1}	0 ($1x10^{-4}$)	0.02	0.04	3.18	$\log_{10}$ (X+min(X))
	Conductivity (Cond)	µS/cm @ 25°C	2.82	229	770	$6.48x10^4$	$\log_{10}$(X)
	Colour (Col)	APHA Pt-Co	0 (1)	20	24.97	840	$\log_{10}$(X+ min(X))
	Disolved organic carbon (DOC)	mg L^{-1}	0.23	5.63	8.83	516	$\log_{10}$ (X)
Physical/ Spatial (NLA 2012)	Lake type (see text for classification details)	binary	Lake: 420; Reservoir: 221; Impoundment: 309				
	Watershed area (A_W)	km^2	0.02	10.2	1762	$6.05x10^5$	$\log_{10}$ (X)
	Surface water temperature (T_S)	°C	9.6	24.2	23.8	36	
	Depth (D)	m	0.1	4	6.6	51	$\log_{10}$(X)
	Day of the year (DOY)	days	129	205	204.2	271	
	Latitude (LAT)		26.07	41.37	40.83	48.99	
	Longitude (LON)		-124.23	-94.93	-95.47	-67.91	
	Urban (lake intersects urban cluster or urban area)	binary	No: 632; Yes: 318				
	Elevation (Elev)	m	0 (0.3)	347.3	661.1	3595	√X
	Lake area (A_L)	ha	1.1	30.04	882	$1.67x10^5$	$\log_{10}$(X)
	Lake area: Watershed area (A_L:A_W)	ha km^{-2}	$2 x10^{-4}$	3.94	8.99	827	$\log_{10}$(X)
	Brunt-Väisälä buoyancy frequency (N)	s^{-1}	$5.14x10^{-6}$	$5.6x10^{-4}$	$7.1x10^{-4}$	$9.86x10^{-3}$	$\log_{10}$(X)

Land use (2012 USDA Cropscape raster with NLA2012 watershed polygons)	Corn (P_c)	fraction of watershed area	0	1.14 x10^{-4}	0.04	0.61	√X
	Corn or Soy (P_{CS})		0	2.78 x10^{-4}	0.07	0.81	√X
	Natural landscape [barren, forest, shrubland, wetlands] (P_{Nat})		0	0.58	0.52	1	√X
	Agriculture [all crops] (P_{Ag})		0	0.17	0.30	0.99	√X
	Developped land [open space to high intensity] (P_{Dev})		0	0.04	0.09	0.98	√X
	Perennial ice/snow and openwater (P_{Wat})		0	0.05	0.09	0.92	√X
(2012 USGS NAWQA County level scale atrazine use estimate data with NLA2012 watershed polygons)	Estimated area density of atrazine application at lake (EA_L)	kg km^2	0 (0.001)	0.255	3.82	51.11	log10(X+ min(X))
	Average estimated area density of atrazine application in watershed (EA_W)	kg km^2	0 (2.98x10^{-5})	0.283	3.97	51.11	log10(X+ min(X))
	Estimated mass of atrazine applied in the watershed (EA_M)	kg	0 (1.08x10^{-4})	3.2	4630	1.89 x10^6	log10(X+ min(X))
Hydrology (Hydrolake 1.0)	Total volume (Vol)	10^6 m^3 (mcm)	0.1	0.169	3.852	1.28 x10^4	$\log_{10}$ (X)
	Discharge average (Dis)	m^3 sec^{-1}	0.001	0.17	3.85	374	$\log_{10}$(X)
	Slope [average within 100m of lake polygon] (Slope)	degrees	0.14	2.27	3.47	24.85	$\log_{10}$(X)
	Shoreline development [ratio between shoreline length and the circumference of a circle of the same area, increases with increasing shoreline complexity] (SDev)	ratio	1.03	1.62	1.96	10.65	$\log_{10}$(X)

Volume reservoir [reported reservoir volume] (VolR)	mcm	0	0	59.03	7815	$\log_{10}$(X+ min(X))
Average depth [ratio between total lake volume and lake area] (DepthAv)	m	0.8	4	7.3	158.9	$\log_{10}$(X)
Residence time [ratio between total lake volume and average discharge] (ResTime)	days	0.1	254	1893	1.99×10^5	$\log_{10}$(X)

5.3.3 Statistical Analyses

To identify the best predictors of atrazine in lakes, we used the modelling strategy adopted by Taranu et al., (2017) consisting of a binomial-gamma [ZAG] hurdle model (also known as delta model). The hurdle model is comprised of two components: 1) A binomial generalized linear model (GLM) identifying the presence or absence of atrazine, and 2) a truncated gamma GLM model predicting the concentration of atrazine above the threshold value when the compound is detected.

a) Hurdle Models

Prior to running the hurdle model (binomial and truncated gamma GLMs), data were transformed and scaled (Table 5.1). We first considered the presence/absence of atrazine above the detection limit through a binomial generalized linear model; coding concentrations below the detection limit as zero and concentrations above as one. Secondly, we considered the log-transformed concentration values after the addition of an offset corresponding to the log transformed value of the detection limit (0.046 μg L^{-1}) (sensu Brilleman et al., 2016); gamma models are defined for a threshold value of zero and this transformation allowed us to consider the detection limit as the hurdle.

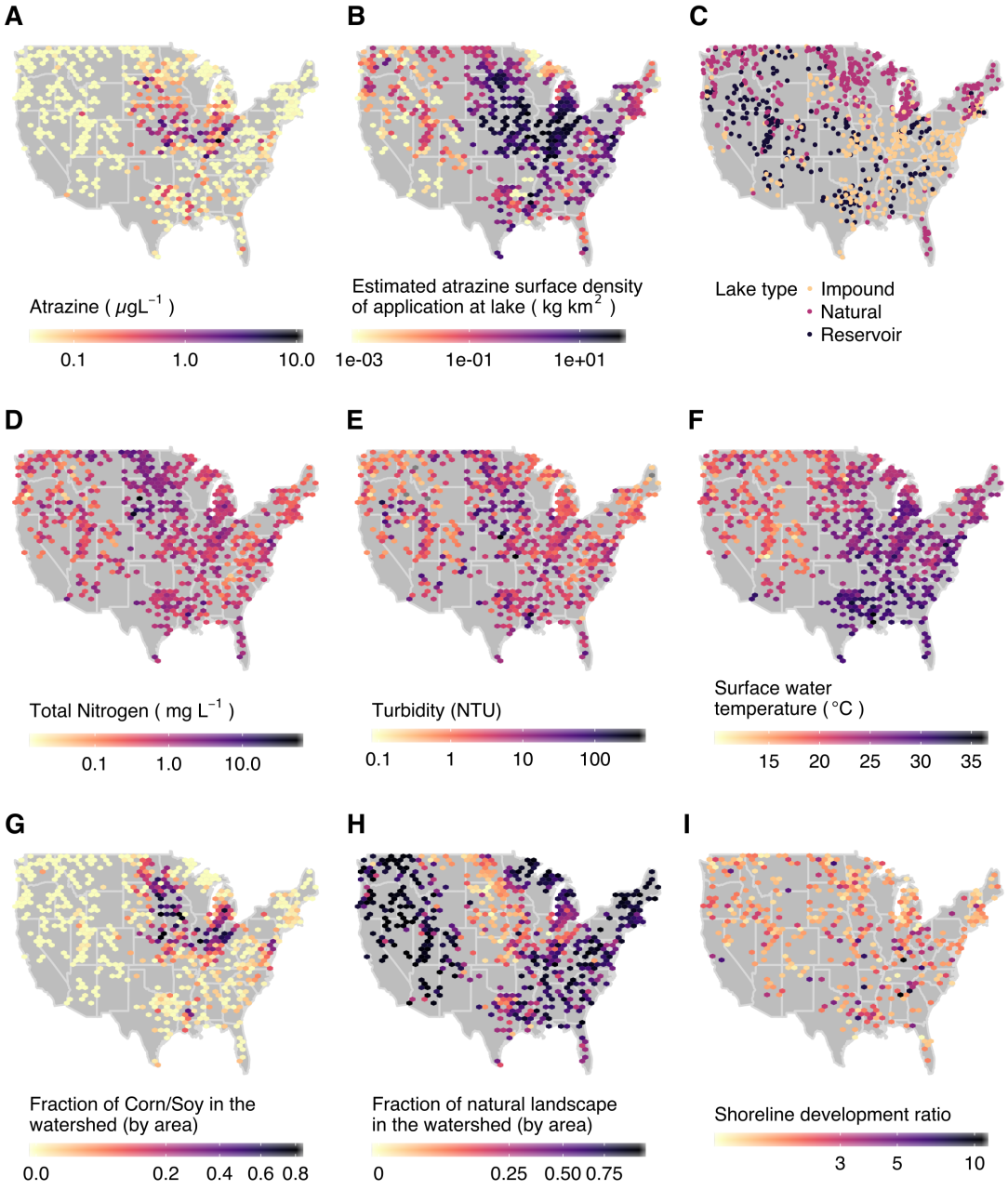

Figure 5.1: Distribution of atrazine and selected variables across the contiguous US. A) Atrazine concentrations from NLA, B) Estimated atrazine application surface density, C) Waterbody type, D-F) Lake properties, G-H) Land-use in the lake's watershed, and I) Lake morphology. To help visualization, for continuous variables (all variables except lake type) the study area was split into 2500 bins (50 vertically and 50 horizontally) the average value within each bin is displayed.

To identify significant predictor variables for both the binomial and truncated gamma GLMs (i.e., parts one and two of the hurdle model), we used the dredge function of the MuMIn R package (Barton, 2018) to generate a subset of 0 to 5 variables from each category (water quality, physical/spatial, land-use, hydrolake), leading to a group of competing models for each category. From the models generated, we retained those that were within three Bayesian information criterion (BIC) units from the best model. While the Akaike information criterion (AIC) is more widely used and also introduces a penalty term for the number of parameters in a model, BIC offers a greater penalty, further limiting the risk of model over fitting, which can be of considerable concern when using model dredging for model selection. For each set of models retained in each variable category, we retained a best model through model averaging, selecting variables with a relative importance greater than 50% based on the sum of "Akaike weights" over all models. Co-linear variables (Pearson's $r>0.5$) were eliminated in competing models and the variance inflation factors (VIF) of the final models were tested to be inferior to 2.

The validation test of the final model residuals could not be conducted by standard methods given our use of GLMs. That is, GLM residuals are not as readily interpreted as for ordinary least-square linear models as the expected distribution of the data changes with fitted values, and standard residual plots cannot aid in determining whether the model is correctly specified. Thus, to test for patterns in the residuals, we used the testResiduals() and simulateResiduals() functions from the DHARMa R package (Hartig 2019) as they simulate scaled residuals from the fitted model. We also computed a pseudo R^2 value considering the proportion of variance explained by the model when compared to the null model.

b) General model

From the variables selected for each category of variables we constructed a general model predicting atrazine occurrence and concentrations across the continental U.S. We felt it necessary to proceed in this matter (first selecting variables within each category, and then building a model joining all) as 1) we were interested in exploring the predictive power of each category, and 2) the number of combinations for model dredging grows exponentially with the number of predictors. Thus, including all variables in a single step was not computationally feasible

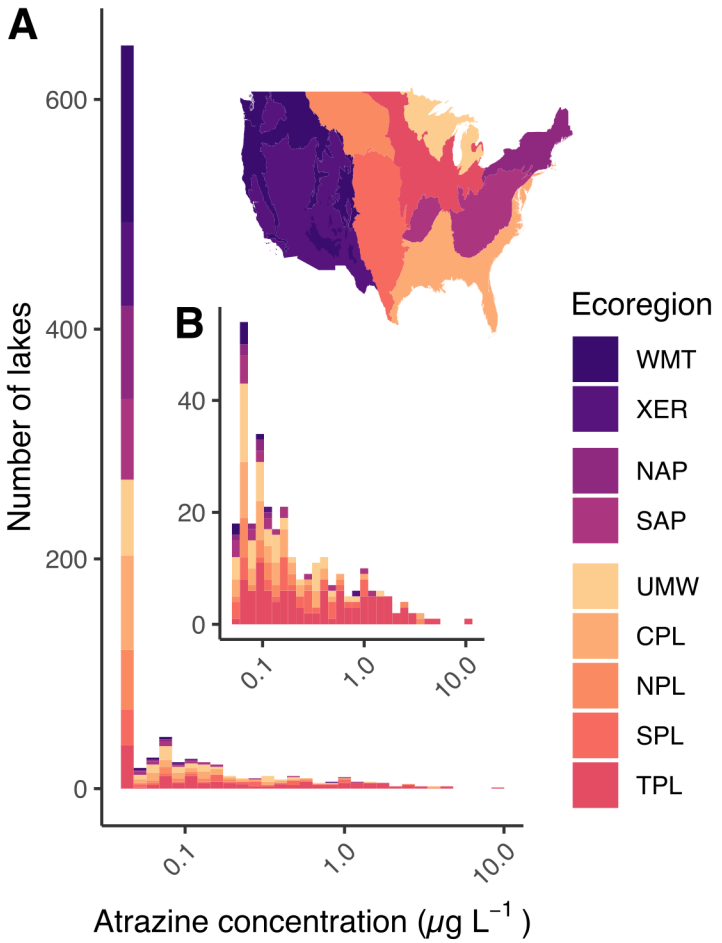

Figure 5.2: Atrazine concentrations in US lakes by ecoregion (ECO9; see Table 5.1 for definition of abbreviations). ECO3 ecolevels (grouped ECO9 levels are as follow: Western mountains and Xeric [dark purple], Eastern highlands [magenta] and Plains and lowlands [orange] (see Table 5.1 for details on ECO9 regions). A) All sampled lakes. B) Subset of lakes where atrazine was found at concentrations exceeding the detection limit.

c) Continental and Plains-Lowlands models

The area sampled by the NLA is divided in National Aquatic Resource Survey (NARS), reporting 3 regions, which are based on aggregated Wadeable Streams Assessment (WSA), reporting 9 regions (Herlihy et al., 2008) (Table 5.1; Fig 5.2: Eastern Highlands [NAP, SAP]; Plains and Lowlands [CPL, NPL, SPL, TPL, UMW]; Western Mountains and Xeric [WMT, XER]). However, based on patterns of atrazine use in North America, we expected atrazine concentrations to be significantly greater in the Plains and Lowlands ecoregions, which comprise the US Corn Belt. Thus, in addition to modelling the continental dataset, we also modelled exclusively for the Plains and Lowlands region, as we hypothesized that variable

selection at the continental scale might be confounded by broad regional differences of the predictive variables considered.

To test whether the two hurdle models (Continental and Plains-Lowlands) were over-fitted to the region used in each analysis, we compared the hurdle model results with a least absolute shrinkage and selection operator (LASSO) regression analysis. LASSO regression is a regulation technique designed to aid generalizing models with complex relationships, such as multicollinearity, through the addition of a penalty to model parameters (Tibshirani 1996). We opted for LASSO regression as it performs variable selection by shrinking coefficients of less important variables to zero (Tibshirani 1996). This however comes at the cost of retaining only a single variable from a group of highly correlated predictors (in contrast, ridge regression retains all parameters, shrinking some close to, but not at, zero). In general, ridge regression works well if there are many variables believed to be of equal importance (when most predictors impact the response), whereas LASSO tends to do well if there are a small number of important variables (when only a few predictors actually influence the response). We used the R glmnet (Friedman et al., 2010) and HDtweedie (Qian et al., 2013) packages for the binomial and gamma parts of the LASSO models, respectively, using a 10-fold cross-validation to fit the models with scaled predictors, extracting the largest lambda value that remained within one standard error of the specified minimum mean cross-validated error (misclassification error for the binomial model and mean square error for the gamma model). We repeated this process 100 times, extracting the mean and standard deviation from the distribution of lambda values. This method allowed us to extract the average relative importance of the variables selected while remaining parsimonious in our selection.

d) Modelling additional atrazine thresholds

We further used LASSO regression to compare the predictors associated with surpassing thresholds other than the limit of detection, as the conditions associated with high atrazine concentrations, for which there is a known environmental concern, might differ from those selected through our gamma models. We considered the 1.8 $\mu g\ L^{-1}$ guideline for the protection of aquatic life (CCME 1999) as well as threshold values of 0.1 and 0.2 $\mu g\ L^{-1}$ atrazine found

to be the maximum maintaining optimum photosynthetic efficiency from our own work (Beaulieu et al., Chapter 4).

5.4 Results

5.4.1 NLA dataset

Atrazine data in the National Lakes Assessment (NLA) dataset is right-skewed and consists mainly of values below the detection limit (Fig 5.2). Atrazine was detected in 32% of the sampled lakes, mainly in the Plains and Lowlands region where the frequency of detection reached 50%. In the Western Mountains and Xeric and Eastern Highlands atrazine was detected in 9% of lakes sampled. When the molecule was detected, concentrations were also lower than in the Plains and Lowlands region ($\bar{x}$=0.18 µg L^{-1}; median=0.08 µg L^{-1} vs $\bar{x}$=0.47 µg L^{-1}; median= 0.15 µg L^{-1} in the Plains and lowlands). Across all lakes sampled by NLA in 2012, the maximum atrazine concentration of 9.7 µg L^{-1} was found in the Temperate Plains ecoregion.

5.4.2 Hydrolake dataset

A total of 761 lakes out of 1135 were used to test for the influence of hydological variables provided in the HydroLAKES (HL) dataset (Annexe A, S5.1). A considerable number of sites (374) did not show overlap of the HL polygons and the NLA sampling sites. While this was in large part due to the 10 ha lake size cut-off value in the HL dataset, 173 of these sites were established as larger than 10 ha in the NLA data. 32 sites additional were rejected due to large discrepancies between variables common to both datasets. We chose to discard sites where differences of over 1.7 orders of magnitude were observed (watershed area, lake area, and elevation). We built two sets of hurdle models, one with the full NLA dataset (1135 lakes) that did not consider hydrological variables and a second (761 lakes) with the reduced dataset considering all predictor categories. This allowed testing the effects of hydrology without discarding the large subset of lakes for which we did not have this data.

5.4.3 Hurdle model – Continental and Plains-Lowlands data

Given that the variable selection method (dredging) used to construct our hurdle models does not allow for missing variables, we constructed the hurdle models using a subset of the data (949 NLA; 568 HL). The total variance explained by the hurdle models (binomial plus gamma parts) ranged from 60% (NLA) to 75% (HL) for the continental scale dataset, and from 46% (NLA) to 66% (HL) for the Plain-Lowlands region (Table 5.2; Fig 5.3). Generalized variance inflation factors (gVIF) never surpassed 1.61 for any of the retained variables. Although reliance on this threshold for variance inflation factors has been criticized (O'Brien 2007), we found that values from our models were well below the value of 10 generally prescribed in the literature (Dormann et al., 2012). This suggests that multi-colinearity is not of great concern for our models. The deviance explained by models based on data limited to the Plains and Lowlands region was generally smaller than that of models considering all ecoregions although the variables retained were generally similar, comprising markers of eutrophication (TN, TP, Turbidity and pH), water temperature, land-use variables and the estimated atrazine application rates. Of the HL variables considered, only Shoreline development (ShD) was found to be a predictor of the presence of atrazine above the detection limit. Based on lasso regression, the proportion of corn and soy culture in the watershed was the most important predictor for the presence of atrazine while, when the molecule was detected, the estimated surface density of atrazine application best explained the concentrations found. The presence of natural landscape was only important in predicting atrazine concentrations, with no effect on atrazine detection. While the estimated application of atrazine in direct vicinity of the lake (EA_L) was a better predictor of atrazine concentrations than the proportion of corn or soy in the watershed (P_{CS}), interchanging these variables generally yielded comparable models as assessed by BIC (Table 5.2). In the HL dataset however, EA_L performed noticeably better. Analysis of the residuals suggests that our models fit the data satisfactorily (Annexe A; S5.2).

Table 5.2: Atrazine hurdle-model of transformed data. Model parameter coefficients with standard errors in parenthesis are presented. Parameters in bold or identified with a star were also selected by LASSO regression. See Table 5.1 for subscript/variable definition and transformation used.

ECO3 Region	Model Component (Predicted variable)	df	β														max GVIF	Pseudo R^2	BIC
			Intercept	P_{CS}	P_{Nat}	P_{Wat}	EA_L	Natural Lake	TN	TP	Turb	Na	Si	pH	T_S	SH_D			
NLA dataset (n=949)																			
All	Binomial (ATR_PA)	945	-5.09 (0.57)	**4.27 (0.42)‡**	*		†‡		**1.74 (0.23)**		*				**0.15 (0.02)**		1.07	0.31	843.6
			-3.24 (0.57)				**0.83 (0.08)**		**1.98 (0.24)**						**0.12 (0.02)**		1.07	0.31	849
	Gamma (logATR-logDL)	297	-2.19 (0.36)	†‡	**-0.85 (0.18)**	*	**0.24 (0.04)‡**	*		0.28 (0.09)	*		†		**0.05 (0.01)**		1.46	0.29	213.9
			-2.67 (0.37)	**0.94 (0.16)**	**-0.86 (0.18)**					0.26 (0.09)					**0.06 (0.01)**		1.45	0.28	216.4
PlnLow	Binomial (ATR_PA)	529	-7.62 (1.57)	**3.49 (0.44)‡**			†‡		*		**0.73 (0.18)**			**0.46 (0.15)**	**0.10 (0.03)**		1.18	0.2	621.6
			-6.43 (1.56)				**0.77 (0.10)**				**0.74 (0.18)**			**0.49 (0.15)**	**0.07 (0.03)**		1.15	0.19	627.7
	Gamma (logATR-logDL)	260		†‡	**-0.80 (0.17)**		**0.23 (0.04)‡**	-0.40 (0.09)			*						1.1	0.26	227
				0.83 (0.16)	**-0.77 (0.18)**			-0.49 (0.09)									1.14	0.25	229.6
HL dataset (n=569)																			
All	Binomial (ATR_PA)	565	-0.79 (0.20)	†‡			**1.13 (0.12)‡**		**1.87 (0.29)**		*				*	2.11 (0.66)	1.03	0.35	496.9
			-1.97 (0.23)	**5.02 (0.55)**					**1.40 (0.28)**							1.92 (0.63)	1.08	0.32	517.8
	Gamma (logATR-logDL)	181		†‡	**-0.89 (0.20)**	**-1.31 (0.39)**	**0.28 (0.05)‡**	-0.43 (0.13)			*		*		*		1.39	0.37	139
				0.86 (0.20)	**-1.00 (0.21)**	**-0.92 (0.41)**		-0.62 (0.13)									1.36	0.35	146.6
PlnLow	Binomial (ATR_PA)	324	-6.96 (1.93)	†‡			**0.90 (0.14)‡**				**0.95 (0.26)**	-0.67 (0.21)		**0.86 (0.24)**			1.48	0.25	371.3
			-6.77 (1.96)	**3.61 (0.57)**							**0.84 (0.25)**	-0.63 (0.21)		**0.74 (0.25)**			1.58	0.23	379.2
	Gamma (logATR-logDL)	160		†‡	**-0.56 (0.23)'**	-1.13 (0.36)	**0.28 (0.05)‡**	-0.52 (0.12)		0.24 (0.11)			-0.25 (0.10)'				1.61	0.43	136
		162		0.84 (0.18)	**-0.85 (0.21)**	-0.87 (0.38)'	**-0.77 (0.12)**										1.37	0.38	138.6

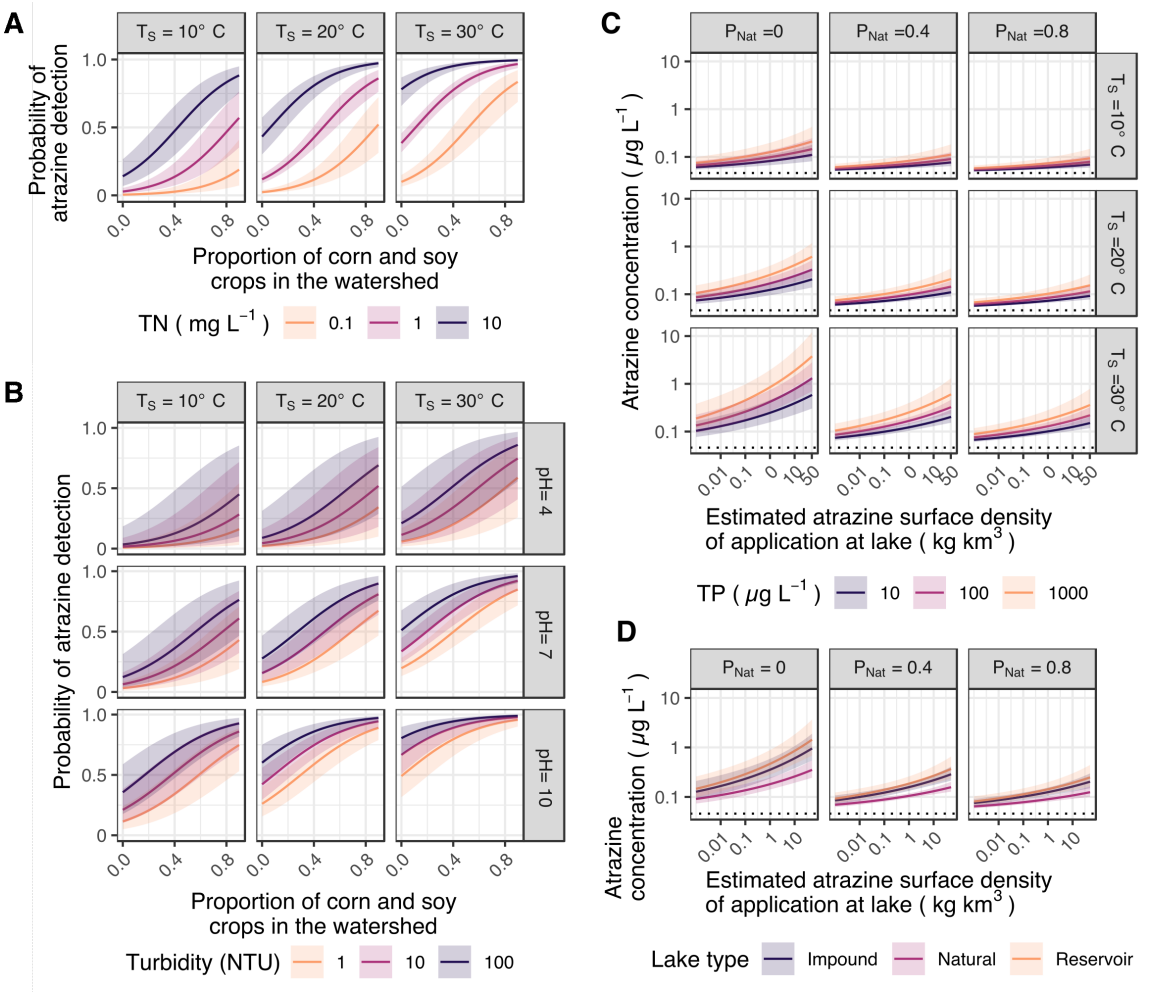

Figure 5.3: Hurdle models fitted curves and confidence intervals. For each panel, the response variable (presence-absence or concentration) is shown on the y axis, while the most important predictor is shown on the x axis, with fixed levels of the other variables represented as panels and colors. A) Binomial component of the model for all ecoregions. B) Binomial component of the model for the Plains and Lowlands ecoregion. C) Gamma model component for all ecoregions. D) Binomial model component for the Plains and Lowlands ecoregion. Atrazine detection limit shown as a dotted line in C and D. See Table 5.1 for details regarding variables.

5.4.4 General model – Continental data

Based on our continental hurdle model we constructed a general model predicting atrazine concentrations across the contiguous US (Fig 5.4A). We found the model to fit the data adequately, although atrazine concentrations in the north of the temperate plains were consistently overestimated while those in the south were underestimated, at times severely (Fig 5.4B).

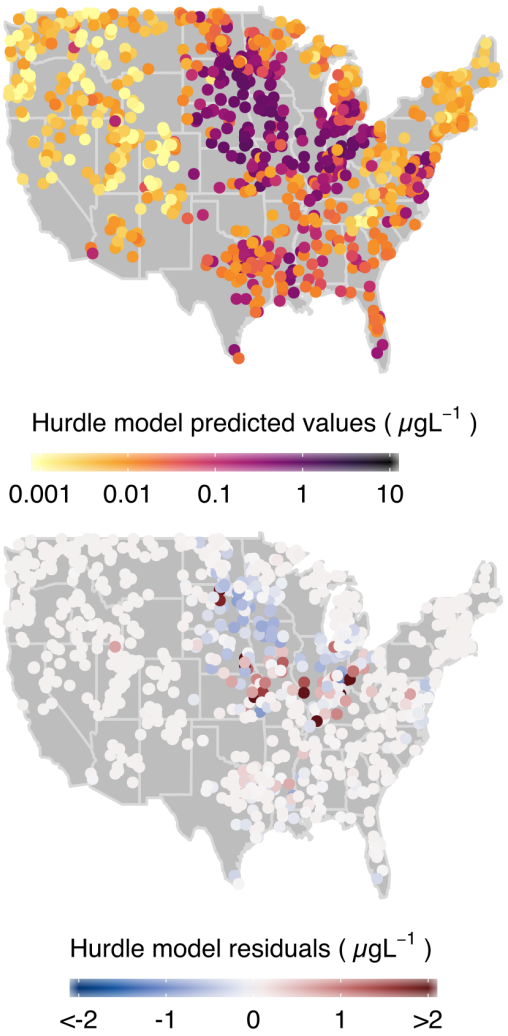

Figure 5.4: General hurdle model performance for continental dataset. The predicted atrazine concentration values are calculated as the probability of binomial success of passing the detection limit (DL) threshold multiplied by the expected gamma value (10^(predicted gamma value +$\log_{10}$(DL) for sites where atrazine was detected and 1 for sites below detect values) A) Atrazine concentrations predicted by hurdle model. B) Model residuals showing an overestimation of atrazine concentrations in the North and an underestimation in the South of the Plains and Lowlands region

Among the water quality variables retained by the general hurdle model, most are indexes of eutrophication resulting from agricultural practices. Increased pH, turbidity, total nitrogen, potassium and silica were found to be predictors in our models, though they were better at explaining the presence than the concentration of atrazine. Among the physical variables tested, only surface water temperature and lake type were selected. Water temperature, especially surface water temperature, can be influenced by eutrophication as increased biomass at the surface of lakes absorbs more heat. Replacing surface water temperature by

average temperature in the water-column still led to the retention of water temperature in the models, although the effects of this variable became weaker. While unimportant for the presence of atrazine, lake type was consistently selected as predictor for atrazine concentration, with lower concentrations in natural lakes than impoundments and reservoirs, which showed no differences.

5.4.5 Hurdle vs LASSO models – Continental and Plains-Lowlands data

The hurdle and LASSO models selected in large part the same variables, although LASSO selected a greater variety of predictors of interest (Fig 5.5). For the NLA dataset (Fig 5.5A), the estimated atrazine applied (EA_L) and the proportion of corn and soy (P_{CS}) remained the best predictors of the detection of atrazine, while the proportion of natural landscape (P_{Nat}) was more important than that of P_{CS} in predicting atrazine concentrations above detection. While ecoregion was not found to be a significant predictor of atrazine concentrations, the presence of atrazine (concentrations greater than the detection limit) was found to be more likely in the Temperate Plains region (ECO[TPL]) for the models run both on all ecoregions and on the subset of lakes within the Plains and Lowlands region. Within the Plains and Lowlands regions, lakes were significantly less likely to have atrazine concentrations above the detection limit in the Northern Plains (ECO[NPL]).

For the HL dataset (Fig 5.5B), only two HL variables were selected with greater shore lengths (SH[L]) being associated with greater probabilities of detecting atrazine and shorter residence times (ResTime) being associated with higher atrazine concentrations. A notable difference between the LASSO models of this dataset and those of the NLA dataset was the importance of the proportion of water in the watershed being negatively correlated with the concentrations of atrazine observed.

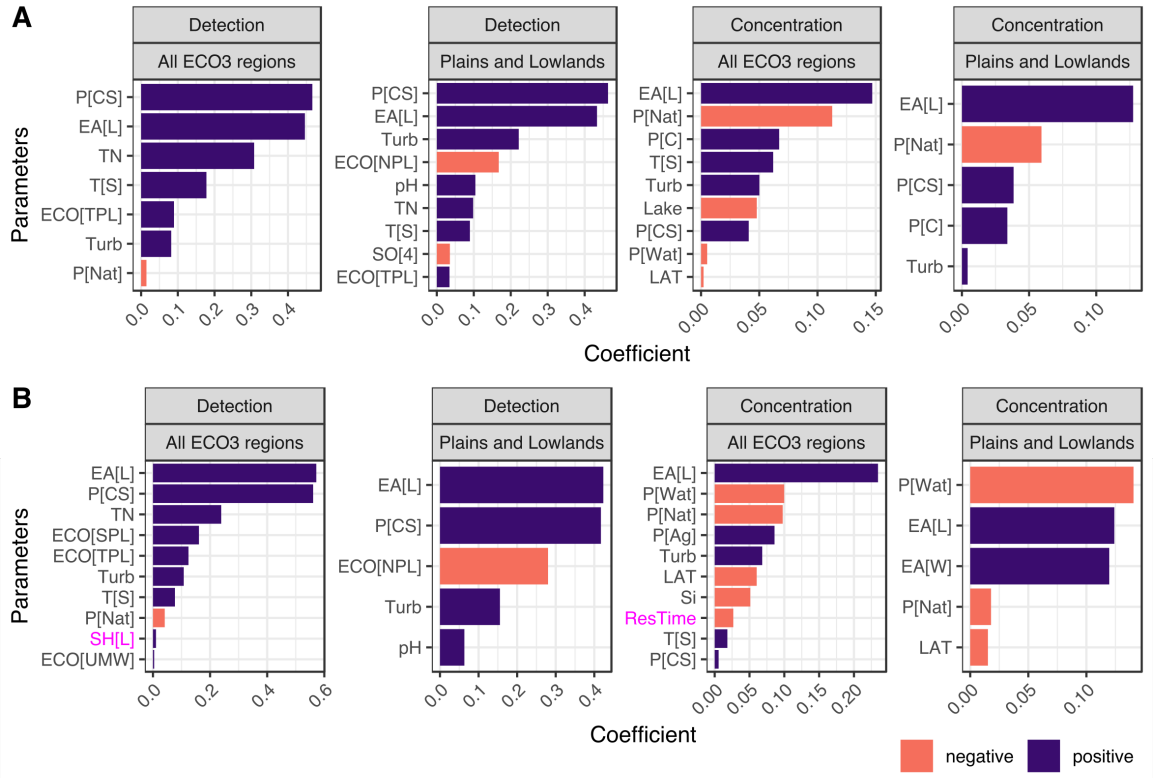

Figure 5.5: Importance of predictor variables as selected by LASSO regression based on coefficients estimated for scaled parameters. A) Models based on NLA dataset. B) Models based on HL dataset (hydroLAKES parameters highlighted in pink). The bar colour identifies whether the value of the coefficient is positive or negative.

5.4.6 LASSO – additional atrazine threshold models

Only seventeen lakes had atrazine concentrations greater than the guideline value of 1.8 µg L^{-1}. We thus chose instead to use a series of smaller thresholds to determine the best predictors of lakes surpassing these limits (DL [302 sites], 0.1 µg L^{-1} [189 sites], 0.2 µg L^{-1} [111 sites], 0.5 µg L^{-1} [63 sites], and 1 µg L^{-1} [37 sites]). As the concentration threshold increased, the deviance explained by our models also increased up to a threshold of 0.5 µg L^{-1}, beyond which the number of sites above the detection limit was likely insufficient (Table 5.3). While being located in the Temperate Plains was always amongst the best two predictors selected, the importance of corn and soy crops was replaced with the type of water body at increasing threshold values, while the importance of the absence of natural landscapes also tended to diminish (Fig 5.6). With regards to HL variables, none were consistently selected.

Table 5.3: LASSO threshold models

Threshold Value	DL (0.046 μgL^{-1})	0.1 μgL^{-1}	0.2 μgL^{-1}	0.5 μgL^{-1}	1.0 μgL^{-1}
NLA dataset (n=949)					
Number sites exceeding threshold	302	189	111	63	37
Optimized lambda	0.05	0.02	0.01	0.01	0.04
Number passes	31	159	237	159	34
Deviance explained	0.30	0.40	0.46	0.48	0.23
Null deviance	1187.25	947.54	684.86	463.47	312.63
HL dataset (n=569)					
Number sites exceeding threshold	187	116	76	44	25
Optimized lambda	0.04	0.02	0.03	0.01	0.06
Number passes	64	173	302	126	25
Deviance explained	0.34	0.49	0.45	0.53	0.20
Null deviance	720.60	575.50	447.36	309.76	205.14

5.5 Discussion

5.5.1 Atrazine and watershed land use

Atrazine is an important contaminant in U.S. lakes, with peak concentrations in the Plains and Lowlands ecoregions. Identifying the main drivers of this heterogeneity is key to helping curtail future increases of and exposure to this harmful contaminant. We show that while the density of surface application of atrazine and land-use within the watershed are the most important determinants of the continued increased concentration of atrazine in lakes, the probability of first observing atrazine above the detection limit was strongly dependant on lakes being located in the temperate plains, with this category gaining in importance when considering higher thresholds. This region has been shown to be especially vulnerable to herbicide transport due to the runoff potential of its soil (Lerch and Blanchard 2003). Atrazine contamination of surfaces waters is greatest in this region with detection in 73% of the lakes sampled and concentrations when detected far superior to dataset averages= 0.79 $\mu g\ L^{-1}$; M = 0.28 $\mu g\ L^{-1}$). While previous studies have suggested that atrazine concentrations in US

Midwest lakes have seen the greatest decreases since the 1990s, comparatively to increases in southern US regions (Yun and Qian, 2015), our results show that this region remains heavily polluted by atrazine.

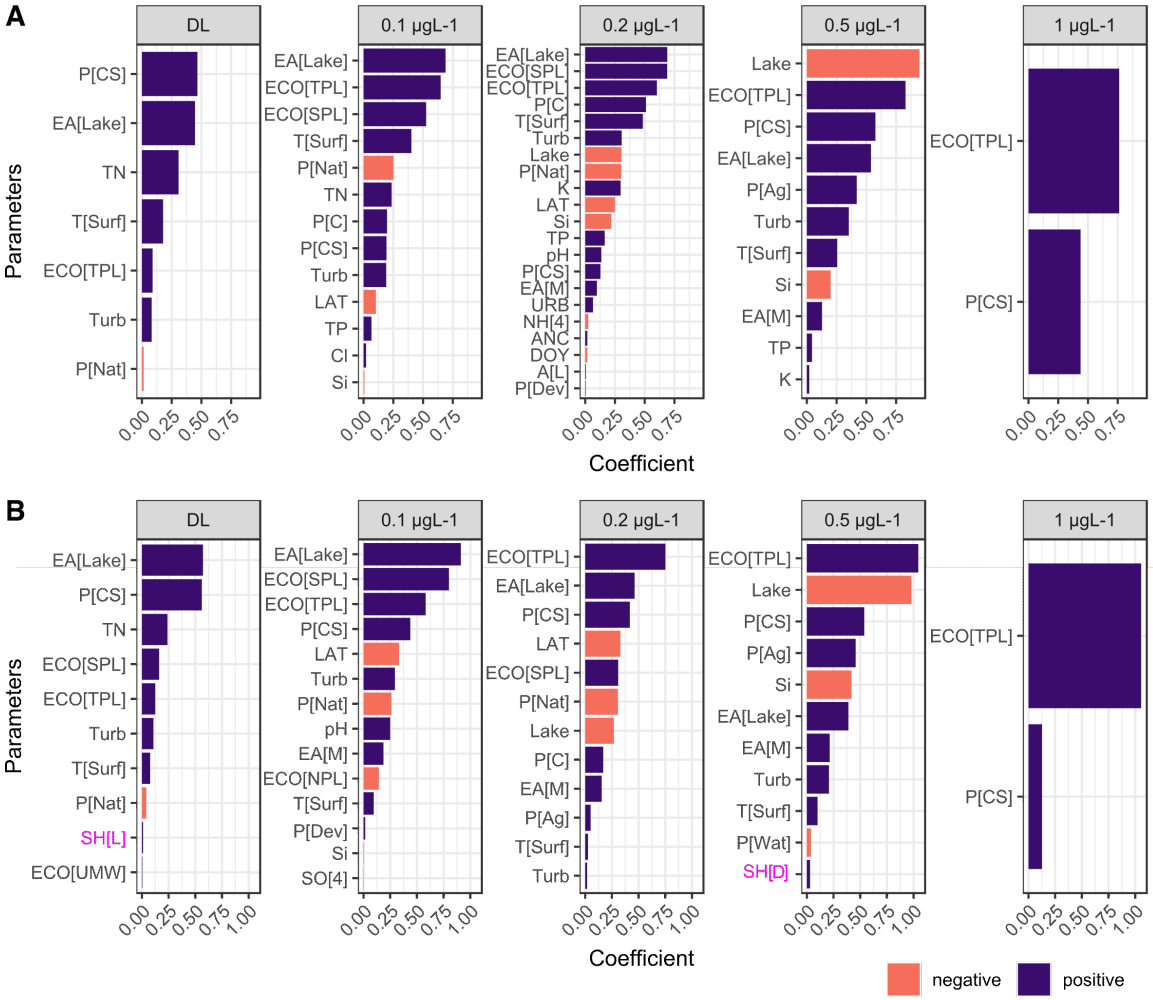

Figure 5.6: Importance of parameters in predicting the probability of surpassing additional atrazine threshold values as selected by LASSO regression. A) Models based on NLA dataset. B) Models based on HL/NLA dataset (HL parameters highlighted in pink). The bar colour identifies whether the value of the coefficient is positive or negative.

Our results demonstrate that knowledge of the area of certain crops within in a lakes' watershed is a suitable replacement for the surface density of application of atrazine. Although the latter was found to be the best predictor of atrazine concentrations in lakes, the former is far more widely available outside of the U.S. The importance of both soy and corn as predictors of atrazine in lakes suggests that the legacy of atrazine use is important in

evaluating atrazine contamination of lakes. While soya crops are often rotated with corn crops (Grassini et al., 2015), soya crops themselves are not treated with atrazine and the importance of this crop in our models is likely reflective of atrazine's persistence in the environment across years, either in soil or in freshwater.

Similarly, natural landscape were the only land cover types consistently associated with the absence of atrazine in lakes, which given the widespread use and persistence of atrazine in the environment, echo the importance of land use activities on atrazine contamination in lakes. Natural landscapes in a lake's watershed may be efficiently retaining and/or removing atrazine from the aquatic environment. While we grouped a number of natural landscapes, it may be of interest to focus on the specific effect of wetlands in the watershed as these landscape features, both natural and constructed, are recognized for their ability to remove pesticides from agricultural runoff (Vymazal and Březinová, 2015). In the Plains and Lowlands region, although natural landscape was not a predictor of atrazine presence-absence, it was an important predictor (negative relationship) of the concentrations found. Thus, despite the substantial atrazine contamination in this region, natural remediation was effective in decreasing concentrations, however, not to levels below the detection limit.

5.5.2 Atrazine and water quality

Although land-use was the most important factor in predicting the presence of atrazine, a significant amount of the variability in the detection of atrazine was accounted for by parameters routinely used to monitor water quality. Unsurprisingly, water quality variables associated with agriculture, erosion and eutrophication, such as total nitrogen, phosphorus, turbidity and pH, were important predictors of the detection of atrazine (Carpenter et al., 1998). However, while soil erosion was not specifically considered, the absence of effect of the slope of the landscape adjacent (100m) to the lake suggests that topography might not play a large role. At the continental scale, lake water chemistry differs significantly between ecoregions and as atrazine is mainly used and detected in the Plains and Lowlands, we found relationships and environmental gradients to be stronger at the continental scale than at the within ecoregion scale, although the predictors remain similar. Interestingly, in contrast to the importance of nitrogen in predicting the presence of atrazine, increased potassium and decreased silica were associated with increasing atrazine concentrations at the continental

scale. Potassium is supplemented in intensive agriculture, and, in lakes, silica loss is generally associated with eutrophication through the uptake of silica and subsequent sinking of diatom frustules. It has also been demonstrated that agricultural harvesting is an anthropogenic sink of biogenic silica, which is highly soluble comparatively to mineral silicates (Vandevenne et al., 2012). This is particularly relevant to the culture of corn and other species from the order Poales as they accumulate substantially more silica in their shoots than other angiosperms (Hodson et al., 2005).

5.5.3 Atrazine and physical variables

In terms of physical variables, surface water temperature was consistently an important predictor at the scales considered, with increased detection of atrazine, and greater concentrations found at higher water temperatures. While increasing temperatures increases elimination kinetics and might be associated with lower concentrations of atrazine, the solubility of the molecule also increases with temperature (Curren and King 2001), which could explain the importance of this physical variable in our models.

Lake type was an increasingly important variable in our models at higher concentration thresholds where natural lakes were associated with smaller concentrations of atrazine compared to reservoirs and impoundments. Subsequent analysis did not reveal substantial difference in the distributions of the water chemistry or physical predictive variables between the lake types considered. While at the continental scale reservoirs were associated with greater proportions of corn and soy cultures, these differences were not significant in the Plains and Lowlands region. Our analysis did not, however, focus on the proximity of corn and soy culture in the watershed to the water body. Natural lakes may be more distal to the cultured land or better buffered from them.

Despite not finding significant effects of hydrological variables in our models, the importance of lake types might reflect hydrological effects. Reservoirs in the United States drain catchment areas on average 6.5 times larger than lakes with residence times half as long (Hayes et al., 2017). Shorter residence times make reservoirs intermediary on the river-lake continuum and, in these systems, increased flow could lead to greater concentrations as the potential of compound transformation decreases. Interestingly, analysis of variance of differences in hydroLAKES variables by lake type followed by Tukey's Honest difference test

showed that natural lakes were characterized by smaller slopes (F-value=9.19, p-value <0.001) and shoreline development ratios (F-value=23.23, p-value <0.001) in the watershed. These results, supported by the inclusion of shoreline development ratios in our models, suggest that the smaller atrazine contamination of US natural lakes may be caused partly by reduced erosion and influence of the surrounding landscape in these systems when compared to reservoirs and impoundments.

5.5.4 Strength of multi-category approach

As agricultural practices are responsible for the contamination of atrazine in aquatic ecosystems, it is not surprising that variables related to agriculture strongly predicted the occurrence of atrazine in lakes. However, physico-chemical properties of lakes as well as catchment hydrology explained an additional and independent portion of the atrazine variability across this broad limnoscape. Remaining, unexplained variability may be due to methodological and fine-scale factors not quantified here. For instance, limitation of analytical methods may fail to detect low atrazine in some lakes, and thus misclassify the lakes as atrazine absent in our binomial models. Furthermore, site-specific differences in atrazine degradation rates may increase noise of the gamma truncated portion of our models, notably since a high degree of variability in atrazine degradation rates has been observed in the literature as a function of land use legacies and the evolution of atrazine-degrading capabilities in the environment (Udiković-Kolić et al., 2012). It has been demonstrated that pre-exposure of soils to atrazine increased the degradation of the molecule over time, as microbial consortia transitioned to organisms capable of transforming the molecule (Houot et al., 2000, Krutz et al., 2010). This suggests that weighing the importance of corn in the watershed by the number of years it has been extensively cultivated in the watershed might improve our ability to predict the presence of atrazine. Our results also support the inclusion of the main metabolites of atrazine in future studies, as this would better characterize the level of contamination in the environment, especially given the known toxicity of atrazine's main metabolites desethylatrazine and deisopropylatrazine (Ralston-Hooper et al., 2009 ; Magnusson et al., 2010, Alberto et al., 2017).

Our models cannot account for the variability in atrazine volumes used on individual farms or their adherence to best management practices, which may prove important. The mechanisms

by which atrazine reaches lakes was likewise not considered in our models, with the exception of watershed slope within 100m of the lake from the Hydrolakes dataset. The inclusion of precipitation-related variables might provide further improvements, as atrazine concentrations in surface waters follow seasonal peaks driven by rain events (Wittmer et al., 2010). Our analysis also overlooked the importance of soil properties; it has been shown that soil runoff potential is a critical factor affecting herbicide transport, with the Temperate Plains region of the US Corn Belt being particularly vulnerable (Lerch et Blanchard 2003). However, fluctuations of the water table within a given field can have great effects on overall losses. Freitas et al., (2008) demonstrated that avoiding atrazine applications on a region corresponding to 1% of a given field could decrease losses by 30%, which suggests that small-scale effects can drive effects at much larger scales, which may never be fully accounted by large continental models. Yet, despite these limitations, the four categories of variables examined in the present study explained as much as 75% of the total variability of occurrence and concentration of atrazine across the contiguous US, with promising findings in terms of avenues for remediation (e.g. natural landscapes).

5.6 Conclusions

- Atrazine contamination of US lakes is well modelled by a binomial-gamma hurdle model.
- After the estimated atrazine surface density of application, land-use variables were the most important predictors of atrazine. The proportion of soy and corn as well as the proportion of natural landscapes in a lake's watershed were predictors of atrazine. Water quality variables related to eutrophication (nitrogen, potassium, silica, turbidity and pH) were also associated with atrazine contamination, as was water temperature.
- While the probability of detecting atrazine did not differ between lakes and impoundments/reservoirs, observed concentrations were significantly lower in natural lakes. This may be influenced by the, on average, smaller shoreline development at these sites, which may be indicative of reduced erosion and influence of the surrounding.

- The ability to predict the presence of atrazine increased at higher threshold values, suggesting that it is easier to predict the presence of atrazine at concentrations representing a greater risk. The Temperate Plains region, likely due to soil properties, is particularly vulnerable to atrazine contamination at higher concentrations.

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CHAPITRE 6

CONCLUSION

6.1 Sommaire

Les besoins en eau des humains sont satisfaits en majeure partie par les eaux de surfaces et les écosystèmes auxquels elles sont liées. La prolifération des efflorescences de cyanobactéries dans les lacs tempérés est une source d'inquiétude pour l'accès à une eau de qualité (Huisman et al., 2018). En plus de causer des problèmes de goût et d'odeur (Watson et al., 2016), et d'épuiser l'oxygène des lacs par la décomposition de leur biomasse, les cyanobactéries sont capables de produire une large gamme de cyanotoxines, dont des hépatotoxines et neurotoxines (Carmichael 1992). La dominance des cyanobactéries dans le phytoplancton des lacs s'est accélérée au cours de l'anthropocène, largement en raison des augmentations des concentrations en nutriments et de la température (Beaulieu et al., 2013 ; Taranu et al., 2015). Les changements climatiques (Betts et al., 2018) et les changements d'affectations des sols peuvent agir de concert pour créer des conditions propices aux efflorescences de cyanobactéries (des effets antagonistes de la température et des nutriments sur les cyanobactéries ont cependant été observés lors d'expériences en mésocosme [Richardson et al., 2019]). Les écosystèmes aquatiques sont des milieux particulièrement vulnérables éprouvants des pertes de biodiversité de loin supérieure à celle des environnements terrestres (Dudgeon et al., 2006). Une des menaces à ces systèmes est la contamination aquatique par des molécules organiques synthétiques. Les herbicides, en raison des organismes qu'ils visent, pourraient être particulièrement susceptibles d'influencer la structure des assemblages de phytoplancton en favorisant les cyanobactéries (Lürling et Roessink 2006).

Les concentrations d'herbicides, ainsi que celles d'une variété d'autres contaminants d'intérêt émergent, ont été quantifiées dans l'environnement. L'atrazine et le métolachlor, deux herbicides, étaient les molécules les plus fréquemment détectées, et ce, à plus grande concentration avec des impacts potentiellement les plus importants pour le phytoplancton en raison de leurs cibles moléculaires (Chapitre 3). Dans les chapitres 1 et 2, nous montrons que des concentrations faibles, mais représentatives des concentrations environnementales de ces herbicides induisent des réponses cellulaires indicatives de stress chez le phytoplancton. Dans le Chapitre 5, nous montrons que les concentrations d'atrazine dans 10 % des lacs aux E-U

sont suffisamment élevées pour affecter la photo-physiologie des communautés naturelles de phytoplancton, selon les critères établis dans le Chapitre 4.

6.2 Contributions

Les effets de l'atrazine et du métolachlor sur le phytoplancton d'eau douce ont été évalués : 1) lors d'expériences longues (3 semaines) sur des communautés naturelles (Chapitre 3) et 2) lors d'expériences courtes (1 semaine) sur des cultures et des communautés naturelles (Chapitre 4). L'utilisation des méthodes moléculaires telles que la fluorescence à répétition rapide (Chapitre 3, Chapitre 4) et l'analyse protéomique (Chapitre 3) ont démontré qu'en l'absence de changements fonctionnels (chlorophylle *a*, Chapitre 3 et 2) et structurels (structure de communautés phytoplanctoniques, Chapitre 3) de faibles concentrations de ces herbicides induisent l'expression d'un plus grand nombre de protéines de choc thermique en présence de métolachlor (HSP70 ; Chapitre 3), alors que l'atrazine (2 $\mu g\ L^{-1}$) affecte le fonctionnement de l'appareil photosynthétique (Chapitre 4).

Les travaux effectués suggèrent que le métolachlor et l'atrazine ont des effets physiologiques sur les communautés aquatiques à des concentrations d'au moins un ordre de magnitude inférieur à ceux prescrits par les lignes directrices pour la protection de la vie aquatique (présentés dans le Tableau 4.3 du Chapitre 4). Le fait de cibler des paramètres physiologiques liés aux modes d'action des herbicides a permis de démontrer leurs effets à des concentrations beaucoup plus faibles que la majorité des études précédentes.

Finalement, des modèles empiriques, prédisant les concentrations d'atrazine dans les lacs des États-Unis contigus, ont été développés (Chapitre 5). Informés par les résultats des effets de l'atrazine sur le phytoplancton (Chapitre 4), en plus d'identifier les meilleurs prédicteurs de la contamination en atrazine, ces modèles peuvent servir à estimer le degré probable de contamination d'un lac donné.

Bien que nos résultats démontrent que les herbicides présents dans l'environnement affectent vraisemblablement la physiologie des espèces de phytoplanctons qui s'y retrouvent, nos résultats n'ont pas démontré des différences de sensibilité entre les algues eucaryotes et les cyanobactéries. Dans le Chapitre 3, nous avons observé une augmentation des isoformes de

protéines de stress uniquement chez les algues eucaryotes, mais l'absence de réponse parallèle chez les procaryotes n'est pas nécessairement indicative d'absence de stress. Dans le Chapitre 4, bien que nous ayons observé que le protocole FRRF fonctionnait de manière optimale pour les chlorophytes et bacillariophytes, les concentrations minimales de réponse étaient similaires pour tous les groupes testés (avec l'exception de *Scenedesmus obliquus* qui semblait plus sensible).

Selon une méta-analyse, il a été proposé que les contaminants organiques persistants (pesticides, pharmaceutiques et produits de soins personnels, polychlorobiphényles et hydrocarbures aromatiques polycycliques) favoriseraient les cyanobactéries (Harris et Smith, 2016). Nous avons décidé d'approfondir cette analyse afin de déterminer si les études supportant cette hypothèse avaient des caractéristiques communes, les distinguant des autres.

6.3 Meta-analyse des contaminants organiques persistants

Harris et Smith (2016) ont établi que les contaminants organiques persistants favorisaient les cyanobactéries par rapport aux algues eucaryotes dans 92 études, comparativement à 61 où les cyanobactéries étaient défavorisées (et 64 où il n'y avait aucune différence). Nous avons étendu leur analyse afin de déterminer si leurs conclusions dépendent des familles de molécules considérées, du type d'expérience et de la durée des expériences. Dans un premier temps, nous avons reconsidéré chaque étude et classifié selon ces catégories (Annexe B) : Concentration effective, essai biologique (où les effets d'une seule concentration étaient testés sur chaque espèce séparément), microcosme (où les tests étaient faits sur des communautés d'algues en laboratoire), mésocosme (essais en enclos extérieurs), étude empirique (comparaison de communautés dans des environnements différant dans leur niveau de contamination). Pour les herbicides nous avons considéré leur mode d'action : Inhibiteurs enzymatiques (regroupant une grande variété de molécules inhibant la croissance ou des processus cellulaires par ce mode d'action), inhibiteurs du photosystème I, inhibiteurs du photosystème II, et Auxines synthétiques. Nous avons également compilé les valeurs de CE50 (Concentration effective réduisant de 50 % la valeur du critère d'évaluation considéré) des études ayant calculé ce paramètre afin de comparer la sensibilité des eucaryotes et procaryotes.

Les herbicides sont les molécules les plus étudiées pour leurs effets sur le phytoplancton (Fig 6.1). Cependant, nous observons que les expériences où des effets positifs ont été observés sont avant tout des expériences où une ou plusieurs concentrations sont testées sur des monocultures d'algues plutôt que des assemblages. Il semble tout de même y avoir un plus grand nombre d'expériences sur des communautés qui démontrent que les cyanobactéries sont plus tolérantes aux herbicides (mésocosme, études empiriques), tandis que les antibiotiques semblent avoir des effets délétères pour les cyanobactéries.

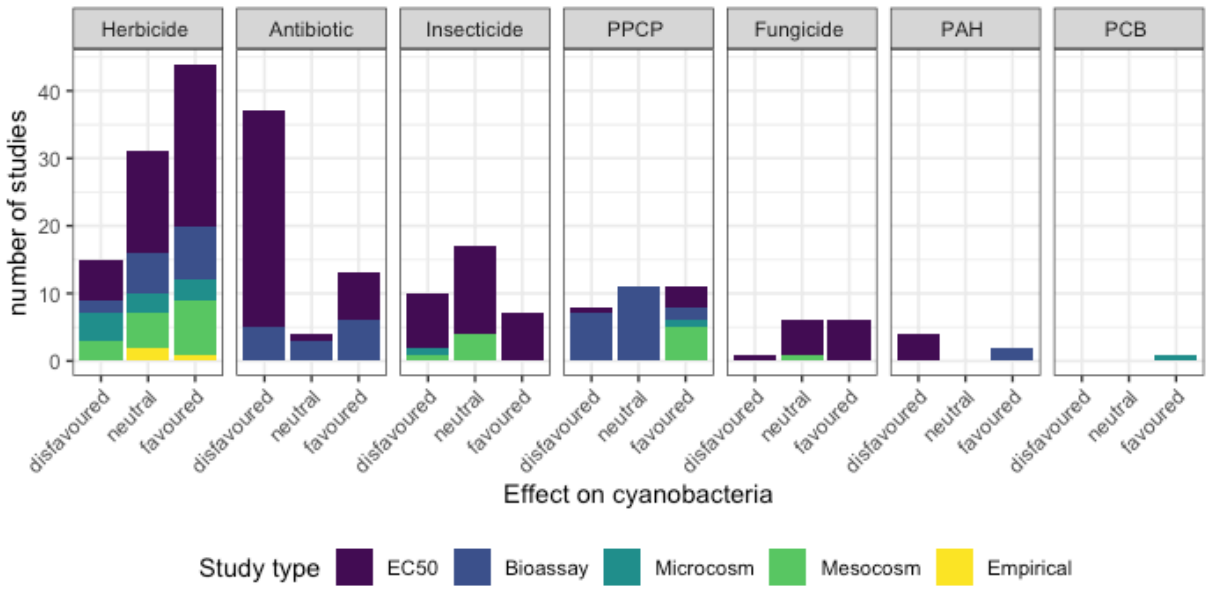

Figure 6.1 : Effets des contaminants d'intérêt émergeant sur les abondances de cyanobactéries relativement aux microalgues eucaryotes. Chaque panneau indique si les cyanobactéries ont été favorisées (favoured), défavorisées (disfavoured), ou si l'effet a été neutre pour différents types de contaminants.

Bien que les études sur des communautés naturelles ou avec davantage de niveaux trophiques sont plus représentatives de la réalité, elles sont également plus difficiles à interpréter, notamment lorsque les résultats sont modulés par les nutriments (Pannard et al., 2009), la saisonnalité (Dorigo et al., 2004) et la température (revue par Gomes et Juneau 2017). La durée des expériences peut également avoir des effets sur les résultats, distinguant les effets chroniques des effets aigus (Kuzmanović et al., 2015). Ainsi, pour les études d'une semaine et plus, on distingue de moins en moins de différences dans la réponse des microalgues eucaryotes et celle des cyanobactéries (Fig 6.2). Les études basées sur des CE50, généralement de courte durée, semblent montrer que les herbicides ont tendance à favoriser les cyanobactéries.

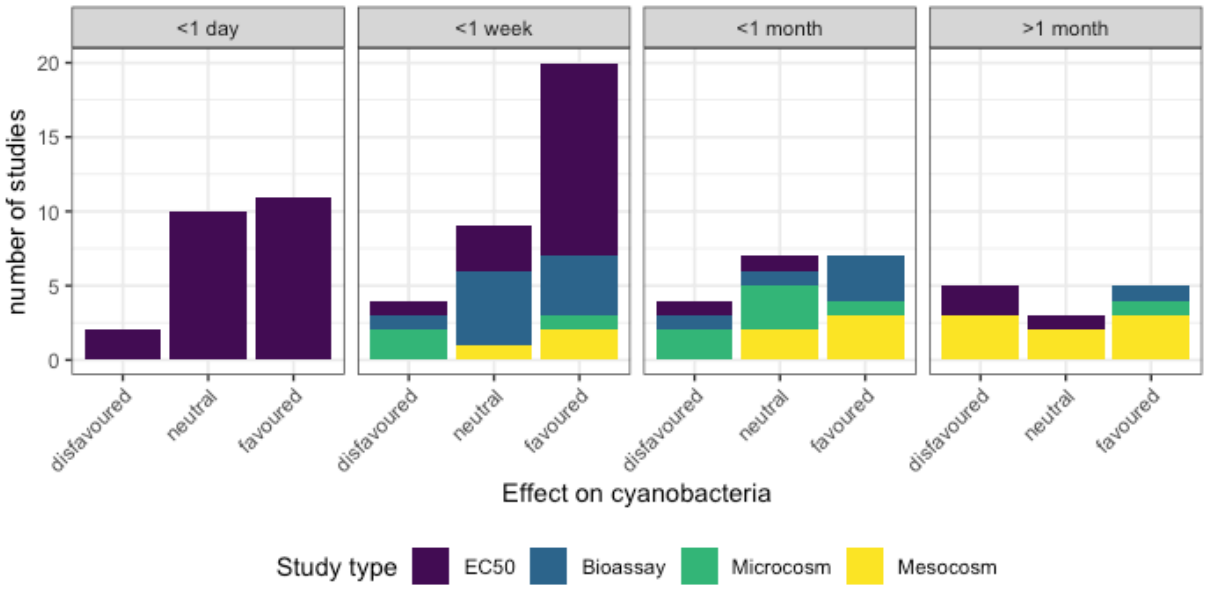

Figure 6.2 : Effets des herbicides sur les cyanobactéries comparativement aux algues eucaryotes selon la durée et le type d'expérience

Le type d'herbicide utilisé pourrait aussi avoir un effet sur les proportions de cyanobactérie par rapport aux algues. Les inhibiteurs du photosystème II et les inhibiteurs enzymatiques variés étaient les types d'herbicides les plus fréquemment testés (Fig 6.3). Alors que les expériences courtes (< 1 semaine) démontraient que ces types d'herbicides favorisent les cyanobactéries, pour les expériences de plus d'une semaine, cet effet n'est qu'observé pour les

inhibiteurs enzymatiques. Le nombre d'études étant plus faible, ces résultats devraient cependant être interprétés avec caution.

Nous observons donc que bien que les cyanobactéries semblent avoir un avantage comparativement aux algues eucaryotes lors d'exposition aux herbicides, ces effets semblent être avant tout aigus, s'estompant pour les expériences d'une semaine et plus, surtout lorsqu'on considère la catégorie des inhibiteurs du PSII.

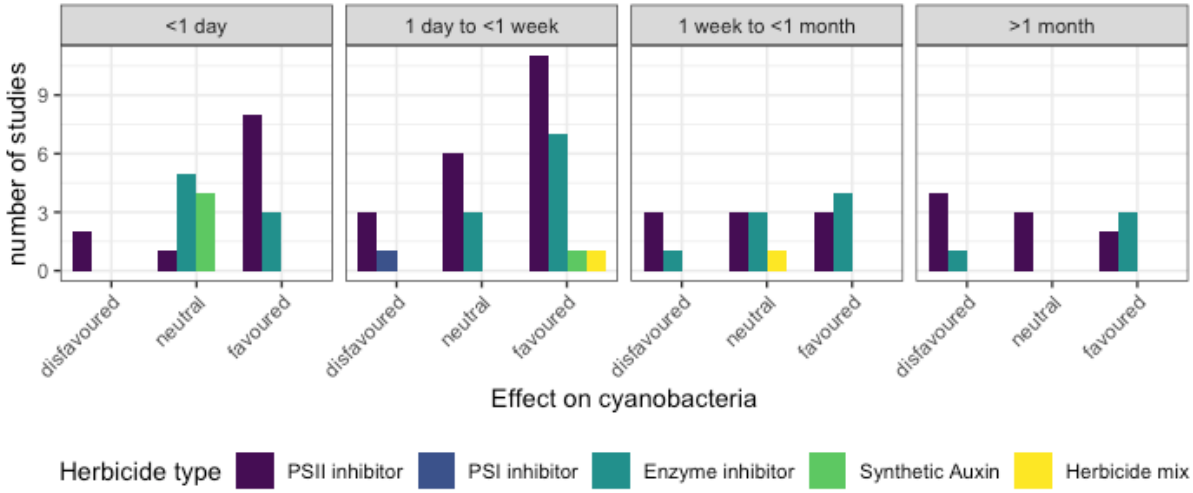

Figure 6.3 : Effets des herbicides sur les cyanobactéries comparativement aux algues eucaryotes selon la durée et le type d'herbicide.

Alors que des différences entre les cyanobactéries et eucaryotes ont été observées dans la littérature primaire constituant cette méta-analyse, nous ne pouvons pas nous prononcer sur l'ampleur de ces différences. Pour ce faire, nous nous reportons aux valeurs de CE50, lorsque présentes, pour 133 cyanobactéries, auxquelles le CE50 est jumelé à celle de la microalgue eucaryote la plus sensible de l'étude présentée (Fig 6.4).

Alors que les herbicides présentaient une grande étendue de concentrations CE50, sans différences entre les deux groupes, une différence fut observée dans la réponse aux antibiotiques (Fig 6.4A). Les cyanobactéries sont des bactéries et leur sensibilité particulière à ces molécules mérite d'être examinée. La présence d'antibiotiques dans l'environnement pourrait réduire la prolifération cyanobactérienne. Alors que nous n'avons pas détecté des concentrations d'antibiotiques élevées dans l'environnement au Chapitre 3, leur présence dans les eaux de surface, parfois à des concentrations de plus de 20 µg L^{-1}, est documentée (Milić et

al., 2013). Bien que le risque le mieux connu des antibiotiques dans l'environnement est l'émergence de gènes de résistance (Pruden et al., 2006), ces résultats indiquent que ces molécules pourraient inhiber les cyanobactéries dans l'environnement. En contrepartie, l'exposition à long terme des cyanobactéries aux antibiotiques pourrait, elle, favoriser la synthèse de microcystines (Wang et al., 2019) ou le développement de résistance.

Lorsqu'on compare les concentrations inhibitives, la méta-analyse ne permet pas de discerner de différence dans la réponse des cyanobactéries et eucaryotes aux herbicides (Fig 6.4A) ni dans leur réponse aux herbicides catégorisés selon leur mode d'action (Fig 6.4B). Conséquemment, s'il existe des différences dans la sensibilité de ces deux groupes aux herbicides lors de courtes expériences de type CE50, elles sont subtiles, et dépendront donc probablement en grande part d'autres facteurs environnementaux. Les concentrations inhibitives plus faibles des inhibiteurs du PSII comparativement à celle des inhibiteurs enzymatiques seraient, elles, possiblement dues au fait que la plupart de ces études avaient une durée de moins d'une semaine, ce qui ne serait pas suffisant afin d'observer les conséquences de l'inhibition des enzymes, comparativement aux changements rapides observés lorsque la photosynthèse est inhibée. On note également que les critères d'évaluations utilisés dans ces études pourraient être biaisés vers l'observation d'effets sur la photosynthèse.

6.4 Perspectives

Selon les travaux présentés dans cette thèse et la littérature existante, les herbicides et autres contaminants d'intérêt émergent peuvent avoir des effets sur les communautés de phytoplancton aux concentrations retrouvées dans l'environnement. Nous n'avons cependant pas trouvé d'évidence que les cyanobactéries bénéficient de la contamination aquatique par les herbicides au-delà d'effets aigus à court terme, ce qui contraste avec les résultats de la méta-analyse concernant les antibiotiques qui favoriseraient les algues eucaryotes. Les nouvelles technologies de la toxicologie des systèmes pourraient cependant éclaircir ce sujet. La transcriptomique serait particulièrement appropriée pour identifier la réponse physiologique à ces agents de stress (Osborn et Hook, 2013), comparant comment ces réponses varient entre groupes.

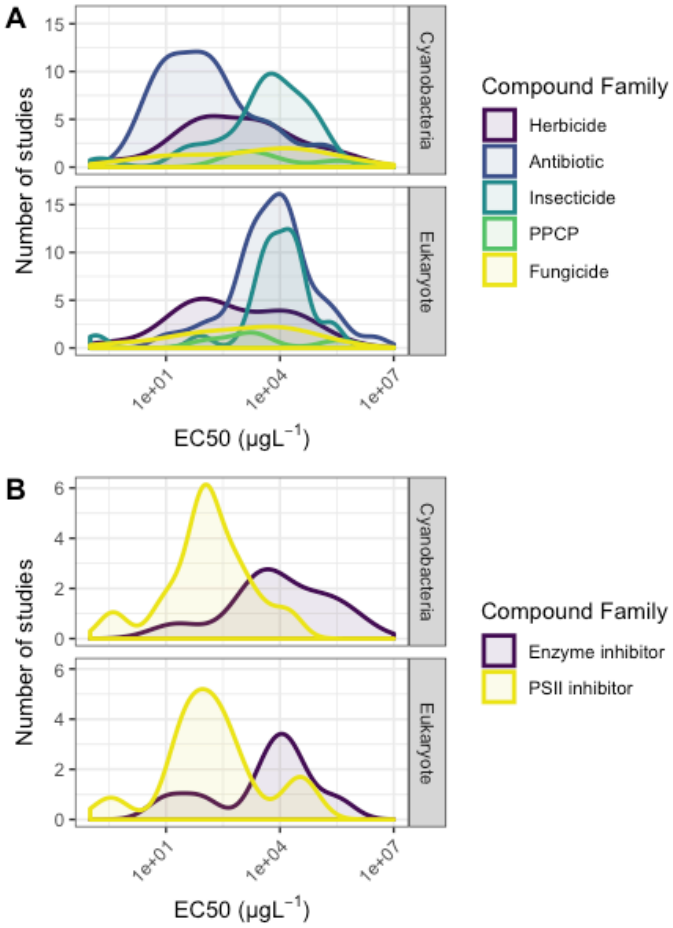

Figure 6.4 : Comparaison des distributions de valeurs d'CE50 entre les cyanobactéries et les algues eucaryotes. Les groupes n'ayant pas assez de représentants pour estimer une distribution furent exclus. A) Comparaison des grandes familles de molécules, B) Comparaison des herbicides selon leur mode d'action.

Bien qu'on ne puisse se prononcer sur la question des cyanobactéries, l'observation d'effets sur le phytoplancton à de faibles concentrations suggère que les recommandations actuelles pour la protection des écosystèmes aquatiques sont insuffisantes pour les herbicides les plus communément détectés dans l'environnement au Québec. L'établissement de standards environnementaux exécutoires serait nécessaire afin d'assurer la protection des milieux d'eau douce et les organismes qui y vivent. La preuve des effets néfastes des pesticides est scientifique, mais tout changement nécessitera un support politique et sociétal, notamment en assurant l'indépendance des chercheurs et des institutions qui gèrent la lutte contre les organismes nuisibles en milieu agricole (Aviv, 2014, Livesey, 2017, Gerbet, T. 2018).

L'investigation des effets environnementaux des herbicides ne remet cependant pas en question les bien-fondés d'une dépendance quasi exclusive aux herbicides pour contrôler les mauvaises herbes. Aucun nouveau mode d'action d'herbicide n'a été découvert depuis 30 ans. Si on considère qu'en raison de développement de résistance aux herbicides, leur efficacité est une ressource épuisable (Davis et Frisvold 2017), la planification et la diversification des mécanismes de protection des cultures doivent être entreprises maintenant, pendant que les molécules actives connues sont encore efficaces (Owen 2016, Davis et Frisvold 2017). L'agriculture biologique diminue les rendements de 5 à 34 % comparativement à l'agriculture conventionnelle, mais les pratiques de diversification et de rotation des cultures limitent ces pertes à 8-9 % (Seufert et al., 2012, Ponisio et al., 2014). Une réduction des applications d'herbicides en tandem avec la rotation des cultures peut diminuer la charge de toxicité aquatique jusqu'à 96 %, et ce, sans diminutions de rendements (Hunt et al., 2017). Outre les rendements, les méthodes alternatives à l'intensification des cultures pourraient avoir d'autres bénéfices socioéconomiques, ceux-ci sont malheureusement peu ou pas documentés (Garibaldi et al., 2017). Ces résultats suggèrent que des investissements en recherche pour ce type de pratiques pourraient permettre de diminuer la dépendance aux produits de lutte chimiques, maintenant des rendements acceptables, tout en protégeant l'environnement.

ANNEXE A

Figures et Tableaux supplémentaires

S4.1: Maximum acceptable toxicant concentration values found based on Dunnetts test and Concentration-response curves for FRRF analysis as compared to chlorophyll a and cell imaging endpoints. MATC values calculated as the geometric mean of the LOEC and NOEC values. All values in µg L^{-1} (NS=Non-significant).

	Minimum Dunnett FRRF parameters			DRC FRRF parameters			PCA			Destructive endpoints		
	first	last	average	first	last	average	first	last	average	Chlorophyll *a*	Particulate area imaged	Particulate mean area
Atrazine (MATC: 17.9 µg L^{-1}; Guideline value: 1.8 µg L^{-1})												
Lakes	3	3	1.1	5.4	4.4	2.9	3	3	1.1			
Cryptomonaea	0.1	1.1	1.1	32.5	15.0	2.5	7	3	3	NS	NS	NS
Cyanophyceae	5	1.1	1.1	62.5	2.8	2.7		3	1.1	11	10	10
Bacillariophyceae	1.1	1.1	1.1	6.9	3.9	1.8	5	3	1.1	NS	NS	NS
Chlorophyceae	0.1	1.1	1.1	2.0	3.6	0.4	0.1	1.1	1.1	NS	3	17
DCMU (MATC: 2 µg L^{-1}; Guideline value: 0.2 µg L^{-1})												
Cryptomonaea	1.1	0.1	0.1	0.7	0.2	0.2	1.1	1.1	0.1	6	6	NS
Cyanophyceae	0.1	0.1	0.1	0.7	0.7	0.3	0.1	0.1	0.1	4	0.1	4
Bacillariophyceae	0.1	0.1	0.1	0.2	0.7	0.3	1.1	0.1	0.1	6	NS	NS
Chlorophyceae	0.1	0.1	0.1	0.4	0.3	0.2	1.1	1.1	0.1	5	5	5
Metolachlor (MATC: 780 µg L^{-1}; Guideline value: 7.8 µg L^{-1})												
Lakes	NS	0.1	11	NS	NS	NS	NS	NS	110			
Cryptomonaea	NS	NS	11	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cyanophyceae	NS	11	11	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bacillariophyceae	NS	1	110	NS	83.7	NS	NS	1	110	110	NS	NS

Chlorophyceae	NS	110	110	NS	163.1	NS	NS	110	110	110	110	110

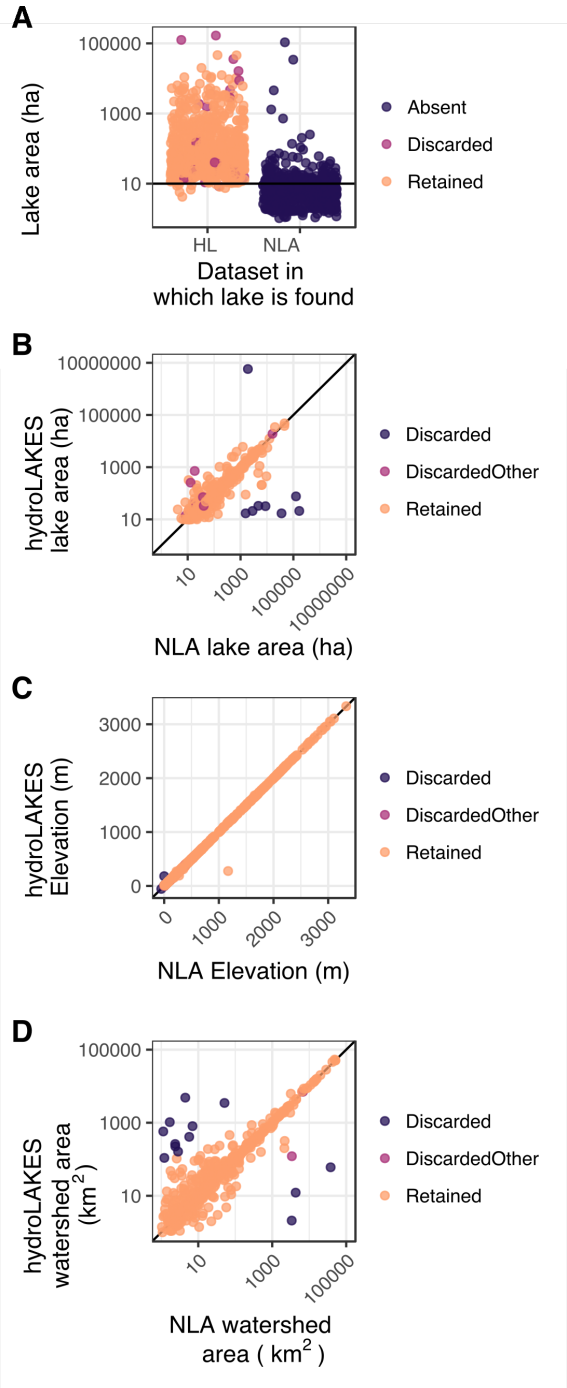

Figure S5.1: Selection of hydroLAKES sites. A) Scatterplot illustrating the omission of lakes smaller than 10ha in the hydroLAKES dataset, as well as a number of smaller, unmatched lakes and few unmatched, larger lakes. B), C), D) Among the lakes geographically matched between the hydroLAKES and NLA datasets, variables common to both datasets were used to discard hydroLAKES sites where differences among variable values differed by more than 1.7 orders of magnitude between datasets (Discarded). Sites discarded for other criteria are also shown (DiscardedOther).

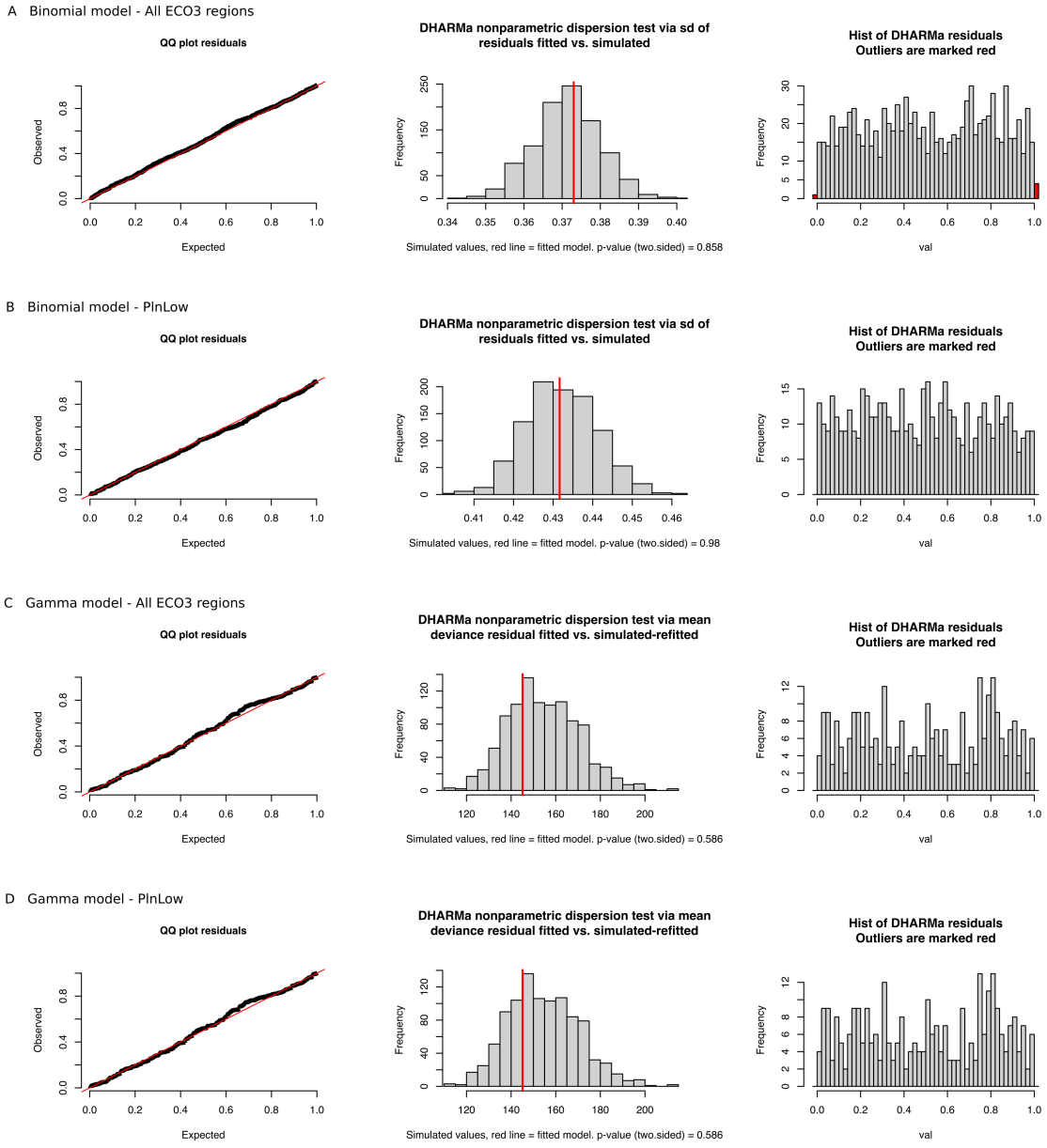

Figure S5.2: Residual plots of NLA hurdle models (HL hurdle models not shown but similar). A) and B) Binomial models. C) and D) Gamma models.

ANNEXE B

Études utilisés dans la méta-analyse

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
					Fongicide					
Ma et al., 2011	Benalaxyl		4	culture	0	EC50	10630	17640	NOEC 1.33 vs 1.62 mgL	
Ma et al., 2011	Chlorothalonil		4	culture	+	EC50	180	130	NOEC 0.018 vs 0.006 mgL	
Ma et al., 2011	Cymoxanil		4	culture	+	EC50	12280	14400	NOEC 0.67 vs 0.9 mgL	
Ma et al., 2004	Fentin acetate		4	culture	-	EC50	16	40	NOEC 0.001 vs 0.005 mgL	
Ma 2005	Fentin-hydroxide		4	culture	0	EC50	20	60	NOEC 0.01 vs 0.004 mgL	
Ma et al., 2011	Fosetyl-aluminum		4	culture	+	EC50	70980	9890	NOEC 23.33 vs 0.58 mgL	
Ma et al., 2011	Hexaconazole		4	culture	0	EC50	3820	1780	NOEC 0.51 vs 0.16 mgL	
Guanzon jr and Nakahara 2002	Isoprothiolane ISP		1	culture	+	EC50	1.13	0.69	0.001 ug/L	Values for photosynthesis, for growth its 1.72 vs 2.55, the opposite trend
Ma et al., 2011	Metalaxyl		4	culture	+	EC50	632439	168310	NOEC 101.67 vs 10.4 mgL	
Ma et al.,2008	Propiconazole		4	culture	+	EC50	19160	1770	0.02 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Abdel-Hamid et al., 1996	Propiconazole		13	phytoplankton	0	Mesocosm	1	NA	1 ugL	When multiple non-cyano species considered always use the lowest concentration for given effect.
Peterson et al., 1994	Propiconazole analytical		1	culture	0	EC50	NA	NA	0.083 mgL	
Peterson et al., 1994	Propiconazole formulation		1	culture	0	EC50	NA	NA	0.083 mgL	
						Herbicide				
Peterson et al., 1994	2-methyl-4-chlorophenoxy acetic acid	Synthetic Auxin	1	culture	0	EC50	NA	NA	1.4 mgL	
Nagai et al., 2013	2,4 Dichlorophenol	Synthetic Auxin	4	culture	+	Bioassay	3300	1500	0.1 mgL	
Peterson et al., 1994	2,4 Dichlorophenoxyacetic aid	Synthetic Auxin	1	culture	0	EC50	NA	NA	2.9 mgL	
Peterson et al., 1994	Acrolein	Enzyme inhibitor	1	culture	0	EC50	NA	NA	1 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Fairchild et al., 1998	Alachlor	Enzyme inhibitor	4	culture	+	EC50	3000	10		
Paule et al., 2013	Alachlor	Enzyme inhibitor	15	periphyton	-	Microcosm	10	NA	10 ugL	
Carder and Hoagland 1998	Alachlor	Enzyme inhibitor	28	periphyton	0	Mesocosm	NA	NA	5 ugL	
Abrantes et al., 2008	Alachlor + Aldrin + Dieldrin + Glyphosate	Herbicide mix	4	culture	+	Bioassay	13.91	NA	ENV	Added environmental water so possible confounding factors though they "corrected" for nutrients, short term assay (96h)
Brain et al., 2012	Atrazine	PSII inhibitor	2	culture	0	Bioassay	56	42.6	5 ugL	NOEC min 5
Chalifour and Juneau 2011	Atrazine	PSII inhibitor	3	culture	0	Bioassay	NA	NA	0.01 uM	
Lockert et al., 2006	Atrazine	PSII inhibitor	5	culture	0	Bioassay	10	NA	0.01 ugL	
Weiner et al., 2004	Atrazine	PSII inhibitor	4	culture	-	Bioassay	44.46	70		
Murdock et al., 2012	Atrazine	PSII inhibitor	6	periphyton	+	Bioassay	71.4	NA	7.7 ugL	
Berard et al., 1999	Atrazine	PSII inhibitor	15	phytoplankton	0	Bioassay	10	NA	10 ugL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Pannard et al., 2009	Atrazine	PSII inhibitor	49	phytoplankton	+	Bioassay	1	NA	0.1 ugL	effects possible but more variable at 0.1 ugL..
Zananski et al., 2010	Atrazine	PSII inhibitor	6	phytoplankton	0	Bioassay	NA	NA	5 ugL	
Abou-waly et al., 1991	Atrazine	PSII inhibitor	7	culture	+	EC50	766	214	70 ugL	
Berard et al., 2003	Atrazine	PSII inhibitor	5	culture	+	EC50	NA	403.41	0.216 ugL	
Deblois et al., 2013	Atrazine	PSII inhibitor	3	culture	-	EC50	22	61	25 nM	Values for quantum yields, electron transfer was 11 nM vs 142 nM
Fairchild et al., 1998	Atrazine	PSII inhibitor	4	culture	0	EC50	90	94		
Peterson et al., 1994	Atrazine	PSII inhibitor	1	culture	+	EC50	NA	NA	2.7 mgL	
Guasch and Sabater 1998	Atrazine	PSII inhibitor	0.04	periphyton	0	EC50	NA	NA	0.05 ugL	EC50 for the entire community comparing light regime different species initially did not look at species at the end of the experiment
Guasch et al., 1998	Atrazine	PSII inhibitor	NA	periphyton	0	Empirical	NA	NA	1 *10-3 umol/L	order of concentrations detected

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Andrus et al., 2013	Atrazine	PSII inhibitor	NA	periphyton/ phytoplankton	0	Empirical	NA	10	ENV	environmental concentrations
Carder and Hoagland 1998	Atrazine	PSII inhibitor	28	periphyton	0	Microcosm	NA	NA	12 ugL	
DeLorenzo et al., 1999	Atrazine	PSII inhibitor	3	periphyton	+	Mesocosm	50	NA	50 ugL	
Herman et al., 1986	Atrazine	PSII inhibitor	84	periphyton	-	Mesocosm	100	NA	100 ugL	
Seguin et al., 2002	Atrazine	PSII inhibitor	30	phytoplankton	-	Mesocosm	30	NA	30 ugL	
Guanzon jr and Nakahara 2002	Chlornitrofen CNP	Enzyme inhibitor	1	culture	+	EC50	0.045	0.018	0.001 ug/L	
Peterson et al., 1994	Chlorsulfuron	PSII inhibitor	1	culture	+	EC50	NA	NA	0.02 mgL	
Sabater and Carrasco 1997	Chlorsulfuron	PSII inhibitor	4	culture	0	EC50	16300	27110	0.07 ppm	
Nystrom et al., 1999	Chlorsulfuron	PSII inhibitor	56	periphyton	-	EC50	268.32675	44112.9177	1 nM	
Abdel-Hamid et al., 1996	Chlorsulfuron	PSII inhibitor	13	phytoplankton	+	Mesocosm	1	NA	1 ugL	
AbdEl-Aty and El-Dib 2009	Cyanazine	PSII inhibitor	4	culture	+	EC50	113	26	7.5 ugL	
Peterson et al., 1994	Cyanazine	PSII inhibitor	1	culture	+	EC50	NA	NA	2.7 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
DeLorenzo et al., 1999	Deethylatrazine	PSII inhibitor	3	periphyton	+	Mesocosm	50	NA	50 ugL	
Peterson et al., 1997	Diquat	PSII inhibitor	1	culture	-	EC50	70	330	3.67 ugL	
Peterson et al., 1994	Diquat	PSII inhibitor	1	culture	-	EC50	NA	NA	0.733 mgL	
Phlips et al., 1992	Diquat	PSII inhibitor	10	culture	-	EC50	122	1272	12 ugL	
Pratt and Barreiro 1998	Diquat	PSII inhibitor	23	periphyton	-	Microcosm	3500	NA	3.5 mgL	
Tadonleke et al., 2009	Diuron	PSII inhibitor	13	bacterioplankton	-	Bioassay	5	NA	1 ugL	picocyano
Pesce et al., 2010	Diuron	PSII inhibitor	NA	periphyton	+	Empirical	4.57	0.31		studying PICT, higher downstream where higher pesticide detection and greater %cyanos
Magnusson et al 2012	Diuron	PSII inhibitor	28	periphyton	+	Microcosm	1.6	NA	1.6 ugL	Diatoms alse increased
McClellan et al., 2008	Diuron	PSII inhibitor	90	periphyton	+	Microcosm	0.08	NA	0.08 ugL	
Leboulanger et al., 2011	Diuron	PSII inhibitor	5	phytoplankton	-	Microcosm	10.82	NA	2.2 ugL	
Villeneuve et al., 2011	Diuron	PSII inhibitor	67	periphyton	0	Mesocosm	NA	NA	2ugL	
Peterson et al., 1994	Ethametsulfuron-methyl	Enzyme inhibitor	1	culture	0	EC50	NA	NA	0.015 mgL	
Ma et al., 2004	Ethephon	Enzyme inhibitor	4	culture	+	EC50	111150	23460	NOEC 30 vs 2 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Ma et al., 2008	Flumetralin	Enzyme inhibitor	4	culture	+	EC50	837170	4620	0.1 mgL	
Caquet et al., 2005	Fomesafen	Enzyme inhibitor	270	phytoplankton	+	Mesocosm	40	NA	40 ugL	
Caquet et al., 2005	Fomesafen / Agral 90	Enzyme inhibitor	270	phytoplankton	+	Mesocosm	130	NA	130 ugL (sum)	
Forlani et al., 2008	Glyphosate	Enzyme inhibitor	28	culture	+	Bioassay	3889	NA	10 uM	
Powell et al., 1991	Glyphosate	Enzyme inhibitor	7	culture	+	Bioassay	845365	NA	5mM	
Vera et al., 2014	Glyphosate	Enzyme inhibitor	8	periphyton	+	Bioassay	3000	NA	3mgL	did NOT analyse community composition, just chl b, could be diatoms
Saxton et al., 2011	Glyphosate	Enzyme inhibitor	2	phytoplankton	0	Bioassay	169.07	NA	1µM	
Lipok et al., 2010	Glyphosate	Enzyme inhibitor	21	culture	0	EC50	164900	292300	0.51 mgL	most cyanos had higher EC50s
Peterson et al., 1994	Glyphosate	Enzyme inhibitor	1	culture	+	EC50	NA	NA	2.9 mgL	
Vera et al., 2010	Glyphosate	Enzyme inhibitor	42	periphyton	+	Mesocosm	8000	NA	8mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Perez et al., 2007	Glyphosate	Enzyme inhibitor	11	periphyton/phytoplankton	+	Mesocosm	6000	NA	6 mgL	
Vera et al., 2012	Glyphosate	Enzyme inhibitor	21	periphyton/phytoplankton	+	Mesocosm	3500	NA	3500 ugL	picocyano
Abdel-Hamid et al., 1996	Glyphosate	Enzyme inhibitor	13	phytoplankton	0	Mesocosm	1	NA	1 ugL	
Stachowski-Haberkorn et al., 2008	Glyphosate	Enzyme inhibitor	7	phytoplankton	0	Mesocosm		NA	1 ugL	
Sun et al., 2013	Glyphosate-isopropylammonium	Enzyme inhibitor	7	culture	+	EC50	1560.773772	NA	1.8 umol	Hormesis effect but not actually compared to other algae
Abou-waly et al., 1991	Hexazinone	PSII inhibitor	7	culture	+	EC50	2752	126	35 ugL	
Peterson et al., 1997	Hexazinone	PSII inhibitor	1	culture	+	EC50	620	29	0.0015 mgL	
Peterson et al., 1994	Hexazinone	PSII inhibitor	1	culture	+	EC50	NA	NA	2.9 mgL	
Peterson et al., 1994	Imazethapyr	Enzyme inhibitor	1	culture	0	EC50	NA	NA	0.067 mgL	
Zhang et al., 2008	Irgarol 1051	PSII inhibitor	4	culture	+	EC50	7.71	0.35		

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Mohr et al., 2008	Irgarol 1051	PSII inhibitor	140	phytoplankton	0	EC50	NA	NA	5 ugL	
Eriksson et al., 2009	Irgarol 1051	PSII inhibitor	15	periphyton	0	Microcosm	NA	NA	3 nM	
Berard et al., 2003	Irgarol 1051	PSII inhibitor	5	phytoplankton	+	Microcosm	2530000	NA	20 ngL	
Daam et al., 2009	Linuron	PSII inhibitor	56	periphyton/ phytoplankton	0	Mesocosm	NA	NA	15 ugL	
Slijkerman et al., 2005	Linuron	PSII inhibitor	30	phytoplankton	-	Mesocosm	20	NA	20 µgL	
Ni et al., 2014	Mesotrione	Enzyme inhibitor	7	culture	+	EC50	6190	4410	0.05 mgL	
Fairchild et al., 1998	Metolachlor	Enzyme inhibitor	4	culture	+	EC50	3000	84		
Peterson et al., 1994	Metolachlor	Enzyme inhibitor	1	culture	0	EC50	NA	NA	3.0 mgL	
Lurling and Roessink 2006	Metribuzin	PSII inhibitor	17	culture	+	Bioassay	100	NA	100 ugL	
Fairchild et al., 1998	Metribuzin	PSII inhibitor	4	culture	+	EC50	100	23		
Peterson et al., 1994	Metribuzin	PSII inhibitor	1	culture	+	EC50	NA	NA	2.7 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Gustavson et al., 2003	Metribuzin	PSII inhibitor	2	periphyton	+	EC50	0.4	NA	0.4 ugL	
Peterson et al., 1994	Metsulfuron-methyl	Enzyme inhibitor	1	culture	+	EC50	NA	NA	0.003 mgL	
Nystrom et al., 1999	Metsulfuron-methyl	Enzyme inhibitor	56	periphyton	-	EC50	19.068	19045.1184	1 nM	
Hartgers et al., 1998	Mixture of Atrazine, Diuron, and Metalochlor	Herbicide mix	28	periphyton/phytoplankton	0	Microcosm	NA	NA	0.54 ; 0.15 ; 0.56 ugL	
Sabater and Carrasco 1998	Molinate	Enzyme inhibitor	4	culture	0	EC50	13100	13420	0.22 ppm	
Leboulanger et al., 2011	Paraquat	PSI inhibitor	5	phytoplankton	-	Microcosm	21.73	NA	5.32 ugL	
Peterson et al., 1994	Picloram	Synthetic Auxin	1	culture	0	EC50	NA	NA	1.76 mgL	
Peterson et al., 1994	Simazine	PSII inhibitor	1	culture	+	EC50	NA	NA	2.7 mgL	
Peterson et al., 1994	Tebuthiuron	PSII inhibitor	1	culture	+	EC50	NA	NA	5.867 mgL	
Peterson et al., 1994	Triasulfuron	Enzyme inhibitor	1	culture	0	EC50	NA	NA	0.018 mgL	
Peterson et al., 1994	Triclopyr	Synthetic Auxin	1	culture	0	EC50	NA	NA	2.6 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
PAH										
Echevest e et al., 2011	Mixture		5	phytoplankton	-	EC50	0.5	NA	0.5 ugL	
Zhu et al . 2012	Mixture of Naphthalene, Phenanthrene, and Pyrene		31	culture	+	Bioassay	536.5	NA	0.486 mgL 0.049 mgL 0.0015 mgL	
De Morais et al., 2014	Pentachloroph enol		10	culture	+	Bioassay	4.41	NA	0.047 ugL	
De Morais et al., 2014	Pentachloroph enol		10	culture	-	EC50	117	5539	0.01 ugL	classified differently than Harris & Smith 2016
Echevest e et al., 2010	Phenanthrene		8	culture	-	EC50	20.8	158	5 ugL (mixed community)	LC10 3.3 vs 24
Echevest e et al., 2010	Pyrene		8	culture	-	EC50	35	675	5 ugL (mixed community)	LC10 2.3 vs 13
PCB										
Kostel et al., 1999	Aroclor 1242		60	periphyton	+	Microcos m	0.001	NA	1 ngL	
Insecticide										
Ma 2005	Azocyclotin		4	culture	-	EC50	40	3780	NOEC 0.0135 vs 0.4612 mgL	
Ma 2005	Beta-cyfluthrin		4	culture	0	EC50	61340	198510	NOEC 8.33 vs 3.12 mgL	
Ma et al., 2006	Carbaryl		4	culture	0	EC50	2490	3934	NOEC 0.4 vs 0.26	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
									mgL	
Peterson et al., 1994	Carbaryl		1	culture	0	EC50	NA	NA	3.667 mgL	
Ma et al., 2006	Carbofuran		4	culture	0	EC50	7950	14670	NOEC 1.67 vs 1.14 mgL	
Peterson et al., 1994	Carbofuran		1	culture	0	EC50	NA	NA	0.6 mgL	
Ma et al., 2006	Carbosulfan		4	culture	+	EC50	187560	28710	NOEC 133.33 vs 1.4 mgL	
DeLorenzo et al., 1999	Chlorpyrifos		3	periphyton	0	Mesocosm	NA	NA	1 ugL	Not included in original study
Tien and Chen 2012	Chlorpyrifos		4	culture	-	EC50	330	1485		
Ma 2005	Cyhexatin		4	culture	0	EC50	30	80	NOEC 0.01 vs 0.01	
Wendt-Rasch et al., 2003	Cypermethrin		14	periphyton/phytoplankton	0	Mesocosm	NA	NA	0.5 ugL	
Ma et al., 2005	Diazinon		4	culture	0	EC50	18320	27450	NOEC 7 vs 2.7 mgL	
Sun et al., 2013	Dimethoate		7	culture	+	EC50	87655.2684	NA	65.63 umol	Hormesis effect but not actually compared to other algae
Abdel-Hamid et al., 1996	Dimethoate		13	phytoplankton	0	Mesocosm	1	NA	1 ugL	
DeLorenzo et al., 1999	Endosulfan		3	periphyton	-	Mesocosm	10	NA	1 ugL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Ma 2005	Fenbutatin-oxide		4	culture	-	EC50	170	2870	NOEC 0.07 vs 0.184 mgL	
Sabater and Carrasco 2001	Fenitrothion		4	culture	-	EC50	1800	4940	0.25 mgL	EC10 de 0.62 vs 2.33 mgL
Leboulanger et al., 2011	Fenitrothion		5	phytoplankton	-	Microcosm	34.66	NA	4.65 ugL	
Guanzon jr and Nakahara 2002	Fenitrothion MEP		1	culture	0	EC50	0.19	0.14	0.001 ug/L	values very close, not exactly clear trend
Ma et al., 2008	Isoprocarb		4	culture	+	EC50	33510	7500	0.05 mgL	mean sensitivity considered
Tien and Chen 2012	Methamidophos		4	culture	0	EC50	2920	6495		
Ma et al., 2005	Methidathion		4	culture	0	EC50	31920	34040	NOEC 5.67 vs 3.5 mgL	
Ma et al., 2006	Metolcarb		4	culture	0	EC50	4200	6850	NOEC 1.57 vs 0.48 mgL	
Wendt-Rasch et al., 2003	Metsulfuron methyl		14	periphyton/phytoplankton	0	Mesocosm	20	NA	1 ugL	fewer chlorophytes at 20ugL
Ma et al., 2005	Phoxim		4	culture	0	EC50	4270	18850	NOEC 0.43 vs 1.9 mgL	
Sun et al., 2013	Phoxim		7	culture	+	EC50	4021.084	NA	1.34 umol	Hormesis effect but not actually compared to other algae
Ma et al., 2008	Propargite		4	culture	-	EC50	98420	251600	1 mgL	
Ma et al., 2006	Propoxur		4	culture	+	EC50	12030	3950	NOEC 3 vs 0.32 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Sabater and Carrasco 2001	Pyridaphenthion		4	culture	-	EC50	5500	12520	0.15 mgL	EC10 de 3.1 vs 8.44 mgL
Tien and Chen 2012	Terbufos		4	culture	0	EC50	700	20875		
Ma et al., 2005	Thionazin		4	culture	-	EC50	4274	25640	NOEC 0.67 vs 1.2 mgL	
Guanzon jr and Nakahara 2002	Tri-n-butyltin chloride TBT		1	culture	+	EC50	0.001	0.00012	0.001 ug/L	
Ma et al., 2004	Triazophos		4	culture	-	EC50	10770	30120	NOEC 2.67 vs 2 mgL	
Sun et al., 2013	Trichlorfon		7	culture	+	EC50	38234.39472	NA	23.35 umol	Hormesis effect but not actually compared to other algae
				PPCP (excluding antibiotics)						
Brezovsek et al., 2014	5-fluorouracil		3	culture	+	EC50	1200	130	20 ugL	
Lawrence et al., 2012	Acetaminophen and Caffeine and Diclofenac		56	periphyton	0	Bioassay	15	NA	5 ugL + 5 ugL + 5 ugL	
Lawrence et al., 2012	Acetaminophen and Diclofenac		56	periphyton	-	Bioassay	10	NA	5 ugL + 5ugL	
Lawrence et al., 2012	Acetominophen		56	periphyton	0	Bioassay	5	NA	5 ugL	
Lawrence et al., 2012	Caffeine		56	periphyton	0	Bioassay	5	NA	5 ugL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Lawrence et al., 2005	Caffeine		56	periphyton	-	Bioassay	10	NA	10 ugL	
Lawrence et al., 2012	Caffeine and Acetaminophen		56	periphyton	0	Bioassay	10	NA	5 ugL + 5ugL	
Lawrence et al., 2012	Caffeine and Diclofenac		56	periphyton	0	Bioassay	10	NA	5 ugL + 5ugL	
Lawrence et al., 2005	Carbamazepine		56	periphyton	-	Bioassay	10	NA	10 ugL	
Brezovsek et al., 2014	Cisplatin		3	culture	-	EC50	670	1520	310 ugL	LOEC concentrations
Lawrence et al., 2012	Diclofenac		56	periphyton	0	Bioassay	5	NA	5 ugL	
Lawrence et al., 2007	Dicolfenac		56	periphyton	-	Bioassay	10	NA	10 ugL	
Lawrence et al., 2007	Dicolfenac		56	periphyton	+	Bioassay	100	NA	10 ugL	
Proia et al., 2013	Dicolfenac		16	periphyton	+	Mesocosm	NA	NA	env	
Pomati et al., 2004	Erythromycin		5	culture	0	Bioassay	NA	NA	1 ugL	
Brezovsek et al., 2014	Etoposide		3	culture	+	EC50	351000	351000	34260 ugL	
Lawrence et al., 2005	Furosemide		56	periphyton	-	Bioassay	10	NA	10 ugL	
Yergeau et al., 2012	Gemfibrozil		56	periphyton	0	Bioassay	1	NA	1 ugL	Compared to other bacteria

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Pomati et al., 2004	Ibuprofen		5	culture	+	Bioassay	1	NA	1 ugL	
Lawrence et al., 2005	Ibuprofen		56	periphyton	-	Bioassay	10	NA	10 ugL	
Proia et al., 2013	Ibuprofen		16	periphyton	+	Mesocosm	NA	NA	env	
Brezovsek et al., 2014	Imatnib mesylate		3	culture	+	EC50	5360	2290	1190 ugL	
Proia et al., 2013	Paracetamol		16	periphyton	+	Mesocosm	NA	NA	env	
Yergeau et al., 2012	Sulfamethoxazole		56	periphyton	0	Bioassay	1	NA	0.5 ugL	Compared to other bacteria
Wilson et al., 2003	Tergitol NP 10		13	phytoplankton	0	Bioassay	NA	NA	0.005 ugL	
Pomati et al., 2004	Tetracycline		5	culture	-	Bioassay	1	NA	1 ugL	
Wilson et al., 2003	Triclosan		13	phytoplankton	0	Bioassay	NA	NA	0.012 ugL	
Drury et al., 2013	Triclosan		34	periphyton	+	Microcosm	10000	NA	10 mgL	Study interested in sediments
Nietch et al., 2013	Triclosan		56	periphyton	+	Mesocosm	0.5	NA	0.1 ugL	
Proia et al., 2013	Triclosan		16	periphyton	+	Mesocosm	NA	NA	env	
						Antibiotic				
Gonzalez pleiter et al., 2013	Amoxicillin		3	culture	-	Bioassay	56300	1500000	EC10 6.16	
Lui et al., LIU et al., 2014	Amoxicillin		7	culture	+	Bioassay	0.8	NA	0.8 ugL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Lui et al., LIU et al., 2012	Amoxicillin		7	culture	+	EC50	8.03	NA		
Lutzhoft et al., 1999	Amoxicillin		7	culture	-	EC50	3.7	3108000		
Halling-Sorensen 2000	Benzylpenicillin (penicillin G) BP		7	culture	-	EC50	6	100000	0.002 mgL	
Halling-Sorensen 2000	Chlortetracycline CTC		7	culture	-	EC50	50	3100	0.002 mgL	
Halling-Sorensen et al., 2000	Ciprofloxacin		3	culture	-	EC50	5	2970		
Robinson et al., 2005	Ciprofloxacin		4	culture	-	EC50	17	18700		
Wilson et al., 2003	Ciprofloxacin		13	phytoplankton	0	Bioassay	NA	NA	0.015 ugL	
Ebert et al., 2011	Ciprofloxacin		3	culture	-	EC50	10.2	8042	1 ugL NOED NA	
Baumann et al., 2015	Clarithromycin		3	culture	-	EC50	12.1	37.1		
Robinson et al., 2005	Clinafloxacin		4	culture	-	EC50	103	1100		
Ebert et al., 2011	Enrofloxacin		3	culture	-	EC50	173	5568	1.6 ugL NOEC 19.1 ugL	
Robinson et al., 2005	Enrofloxacin		4	culture	-	EC50	49	3100		

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Gonzalez pleiter et al., 2013	Erythromycin		3	culture	-	Bioassay	22	350	EC10 0.005	
Wan et al., 2015	Erythromycin		8	culture	+	Bioassay	0.001	NA	0.001 ugL	Inhibited at higher concentrations
Yergeau et al., 2012	Erythromycin		56	periphyton	0	Bioassay	1	NA	1 ugL	Compared to other bacteria
Robinson et al., 2005	Flumequine		4	culture	-	EC50	1960	5000		
Lutzhoft et al., 1999	Flumequine		7	culture	-	EC50	159	18000		
van der Grinten et al., 2010	Flumequine		1	culture	-	EC50	8800	16000		
Wan et al., 2014	Levofloxacin		7	culture	+	Bioassay	0.001	NA	0.001	Inhibited at higher concentrations
Robinson et al., 2005	Levofloxacin		4	culture	-	EC50	7.9	7400		
Robinson et al., 2005	Lomefloxacin		4	culture	-	EC50	186	22700		
Halling-Sorensen et al., 2000	Mecillinam		3	culture	-	EC50	60	300000		
Stoichev et al., 2011	Minocycline		12	culture	+	EC50	420.87884	NA	0.04 µM	EC20 0.13 µM, enhancement due to more stable degradation products as enhancers
Gonzalez pleiter et al., 2013	Norfloxacin		3	culture	-	Bioassay	5600	80000	EC10 1.2	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Robinson et al., 2005	Oflaxacin		4	culture	-	EC50	21	12100		
Halling-Sorensen 2000	Olaquindox O		7	culture	-	EC50	5100	40000	0.5 mgL	
Lutzhoft et al., 1999	Oxolinic acid		7	culture	-	EC50	180	10000		
Kolar et al., 2014	Oxytetracycline		3	culture	+	EC50	2700	1040	EC10: 0.47 mgL vs 1.5 mgL	
Lutzhoft et al., 1999	Oxytetracycline		7	culture	-	EC50	207	1600		
van der Grinten et al., 2010	Oxytetracycline		1	culture	+	EC50	5400	600		
Gonzalez pleiter et al., 2013	Quinolones levofloxacin		3	culture	-	Bioassay	4800	120000	EC10 1.1	
Lutzhoft et al., 1999	Sarafloxacin hydrochloride		7	culture	-	EC50	15	24000		
Liu et al., 2014	Spiramycin		7	culture	-	Bioassay	0.8	NA	0.8 ugL	Not included in Harris and Smith 2016
Lui et al., 2012	Spiramycin		7	culture	-	EC50	1.15	NA		
Halling-Sorensen 2000	Spiramycin SP		7	culture	-	EC50	5	2300	0.002 mgL	

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Qian et al., 2012	Streptomycin		4	culture	-	EC50	280	20080	50 µgL	
van der Grinten et al., 2010	Streptomycin		1	culture	-	EC50	34	1500		
Halling-Sorensen 2000	Streptomycin ST		7	culture	-	EC50	7	133	0.002 mgL	
Lutzhoft et al., 1999	Sulfadiazine		7	culture	-	EC50	135	403000		
Yergeau et al., 2012	Sulfamethazine		56	periphyton	0	Bioassay	1	NA	0.5 ugL	Compared to other bacteria
van der Grinten et al., 2010	Sulphamethoxazole		1	culture	-	EC50	550	9000		
Gonzalez pleiter et al., 2013	Tetracycline		3	culture	+	Bioassay	6200	3310	EC10 2.5	
Yang et al., 2013	Tetracycline		7	culture	+	Bioassay	200	NA	50 ugL	
Halling-Sorensen 2000	Tetracycline TC		7	culture	-	EC50	90	2200	0.003 mgL	
Halling-Sorensen 2000	Tiamulin TI		7	culture	-	EC50	3	165	0.0025	
Halling-Sorensen et al., 2000	Trimethoprim		3	culture	0	EC50	112000	110000		

Study	Contaminant	Contaminant group (for Herbicides)	Duration of study (days)	Organisms tested	Response of cyanobacteria	Experiment type	EC50 cyanobacteria (µgL-1)	EC50 eukaryote (µgL-1)	Lowest concentration tested	Notes
Kolar et al., 2014	Trimethoprim		3	culture	+	EC50	253000	129000	EC10: 26 mgL vs 65 mgL the opposite than EC50	
Lutzhoft et al., 1999	Trimethoprim		7	culture	+	EC50	112000	16000		
van der Grinten et al., 2010	Trimethoprim		1	culture	-	EC50	6900	9000		
Pinckney et al., 2013	Tylosin		10	periphyton	+	Bioassay	10	NA	0.011 umolL	
van der Grinten et al., 2010	Tylosin		1	culture	+	EC50	290	8.9		
Halling-Sorensen 2000	Tylosin TY		7	culture	-	EC50	34	1380	0.002 mgL	

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